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Measurement of Biofilm in A Biological Drinking Water Treatment Plant

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BY

Pu Lu

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

DEPARTMENT OF CIVIL ENGINEERING
EDMONTON, ALBERTA
FALL, 1993

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Pu Lu
11703 - 37B Ave
Edmonton, Alberta
Canada T6J 0K5

Sept 30 19 93

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Measurement of Biofilm in a Biological Drinking Water Treatment submitted by Pu Lu in partial fulfillment of the requirements for the degree of Master of Science.

.....

Dr. P. M. Huck

Supervisor

.....

Dr. D. W. Smith

Co - Supervisor and Chair

.....

Dr. P. M. Fedorak

.....

Dr. G. R. Finch

Sept 14/93

ABSTRACT

Biological processes are receiving increasing interest as a means of drinking water treatment in North America. The implementation of biological processes will be facilitated if tools can be developed to predict their performance under various operating conditions and raw water qualities. A pilot plant research project funded by the American Water Works Association Research Foundation is examining whether biofilm process fundamentals and kinetics largely developed for wastewater treatment can be applied to the design and operation of biofilm processes in drinking water treatment. Important parameters required in this regard are the thickness and density of the biofilm on sand, anthracite or granular activated carbon (GAC) in biological filters or GAC contactors. This thesis reviews various methods investigated to measure these parameters and presents and interprets results obtained with the methods selected.

Several techniques involving scanning electronic microscopy (SEM) were evaluated. SEM combined with critical point drying caused serious shrinkage of the biofilm. SEM combined with freezing the sample provided a relatively true picture of the biofilm. A method which estimates biofilm thickness by measuring loss on drying.

To obtain the density of the biofilm, biomass has to be measured. A thermogravimetric analysis method developed for wastewater was not sensitive enough to measure the very low amount of biomass present. However, a method involving lipid phosphate determination using malachite green provided accurate measurement of the biomass.

So, the SEM method with freezing, and the method involving lipid phosphate determination using malachite green were selected to measure the thickness and biomass of the biofilm. The measured biomass by the phospholipid method was expressed as nmol P/g media. The biomass measurements also have been converted to nmol P/cm^3 reactor

and number of bacteria/g media by multiplying the nmol P/g media values by the density of the media and the conversion factor between nmol P and number of bacteria, respectively.

The selected methods were used to determine the amount and thickness of biomass in filters and GAC contactors in the pilot plant referred to above.

Generally, biomass decreased along the depth of filters or contactors receiving preozonated and non pre-ozonated water, but in the filter receiving prechlorinated water, the biomass increased along the depth of the filter. Prechlorination and preozonation increased the amount of biomass in the filter/contactor compared to the filter/contactor receiving non-prechlorinated and nonpreozonated filter/contactor. In the top layer of the filter, the amount of biomass in the filter receiving preozonated water was higher than the amount of biomass in the filter receiving prechlorinated water. More biomass was present in the filter operating with the higher hydraulic loading than in the filter operating with the lower hydraulic loading. GAC was a better biomass support medium than anthracite, but this was mainly caused by the larger surface area of GAC. GAC was a better medium than sand, however this may not be caused by the larger surface area of GAC. Backwashing decreased the amount of biomass significantly.

The amount of biomass in the GAC contactors was linearly related to the input assimilable organic carbon (AOC) concentration, and the amount of AOC removal in the GAC contactors was also linearly correlated with the amount of biomass. The thickness estimated from SEM photographs provided a good indicator of the amount of biomass, but shrinkage of biofilm still occurred during the sample preparation. Heterotrophic plate counts after the dual media filters increased significantly compared to the levels before the dual media filters, but this phenomenon did not occur in the GAC contactors.

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LIST OF ABBREVIATIONS

GAC: granular activated carbon

SEM: scanning electron microscopy

AOC: assimilable organic carbon

HPC: heterotrophic plant count

VSS: volatile suspended solids

ESEM: environmental scanning electronic microscopy

ADOC: acridine orange staining

EPS: extracellular polymeric substance

COD: chemical oxygen demand

SDS: sodium dodecyl sulfate

TCA: trichloroacetic acid

TGA: thermogravimetric analysis

ThOD: theoretical oxygen demand

DAPI: 4', 6-diamidino-2-phenylindole

NVOC: non volatile organic carbon

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Chapter 1 INTRODUCTION

Biological treatment processes are methods in which the removal of contaminants is by biological activity. Microbial cells attach firmly to the surface of support media, grow, reproduce and produce extracellular polymers which frequently extend from the cell forming a tangled matrix of fibers which provide structure to the assemblage. The immobilized cells and the organic polymer matrix form the biofilm (Christensen, 1989).

Biological processes have historically played a role in drinking water treatment through the use of slow sand filters. In the last 10 to 20 years that they have begun to receive attention once again for removal of ammonia and organic carbon without causing harmful disinfection by-products formation.

The renewed application of biological processes and the accompanying research has occurred primarily in Western Europe, in part because of poorer surface water quality than in North America, but also because of stricter regulations and greater aesthetic demands of consumers. Instead of using biological processes, North American utilities usually introduce a disinfectant as early as possible in the treatment process. As consequence microbiological activity in the treatment plant is minimized. Therefore there is little or no opportunity to remove organic matter in the treatment process prior to disinfection, and therefore levels of disinfection byproducts are higher than otherwise.

Some disinfection byproducts have been found to be of health concern. For example: chloroform is carcinogenic to animals (Bull et al., 1991), 3-chloro-4[dichloromethyl]-5-hydroxy-2[5H]-furanone is mutagenic (Christman et al., 1991). Because of their ability to control organics and minimize the byproducts of disinfection, biological processes are receiving more attention in North America as a potential treatment to meet stricter regulations for organic substances, especially disinfection byproducts.

The implementation of biological processes can be facilitated if tools can be developed to predict their performance under various operating conditions and raw water qualities. A research project funded by the American Water Works Association Research Foundation is examining whether biofilm process fundamentals and kinetics largely developed for wastewater treatment can be applied to the design and operation of biofilm processes in drinking water treatment. This investigation is being conducted largely at pilot scale, using a pilot plant located at the Rossdale Water Treatment Plant in Edmonton, Alberta. This pilot plant obtained raw water from the North Saskatchewan River. Treatment commenced with screening, presedimentation, coagulation, flocculation and clarification, following which the flow was split into several parallel streams. Part of the flow went directly to chlorinated and non-disinfected control filters, while the remainder was ozonated before being fed to three parallel filters with different media and operating conditions. All of the filters except the chlorinated control were followed by biologically active GAC contactors. Samples obtained from the pilot plant were analyzed for assimilable organic carbon (AOC) and various chemical parameters. Kinetic models were applied to the data from the biofilters. Important parameters required in this regard are the thickness and density of the biofilm on sand, anthracite or GAC. This thesis reviews various methods to measure these parameters, and presents and interprets results obtained with the methods selected.

Chapter 2 OBJECTIVES

The specific objectives of this thesis are: (1) to review methods that could be used to measure the thickness and amount of the biofilm, (2) to select methods for initial evaluation, (3) to assess their efficiency by measuring the thickness and the amount of biofilm attached to different media in the laboratory or pilot plant, (4) to measure the thickness and amount of biofilm attached to sand, anthracite and GAC in the pilot plant using the most suitable method, (5) and to interpret the results obtained with the methods.

Chapter 3 LITERATURE REVIEW

In the past ten years, a lot of work has been done to measure the thickness and the biomass of the biofilm in wastewater treatment. Because of the high concentration of organic matter, a very thick biofilm usually develops. Thickness can be measured by using light microscopy, and biomass (the mass of active or living biofilm) can be measured by using the standard volatile suspended solids (VSS) method or a method specific for measuring cellular material such as ATP, protein, carbohydrates or organic nitrogen. Since the concentration of organic substances present in drinking water is much lower than that found in wastewater, a much thinner biofilm will be expected. Therefore, methods with high sensitivity have to be found to measure the thickness and biomass of the biofilm on the media in drinking water treatment.

3.1 Methods for Measuring the Thickness of Biofilm

Several methods have been developed for measuring biofilm thickness based on microscopy techniques, porosity of the porous media and moisture content of the biofilm.

Scanning electron microscopy (SEM) has been used to examine the physical and structural characteristics of biological growth on support media such as activated carbon. It is preferred over conventional light microscopy because of its greater magnification, increased depth of field, and higher resolution and three-dimensional image (Weber et al., 1978).

Different sample preparation methods for SEM have been developed to get a vivid picture of the biofilm or bacteria. However, the general procedure is almost same for each case; samples are fixed, washed in buffer, postfixed, dehydrated in ethanol, then dried, mounted on a stub, sputtered with gold. Two different sample drying methods have been used: one is critical point drying and another is freeze drying. Different fixing solutions have been used in each case.

Allmen et al. (1982) used SEM to observe the biofilm attached to a rotating biological contactor. Samples were immediately fixed with 2.5% glutaraldehyde in Millonig's buffer for several hours. Subsequent treatment included a 10 min buffer rinse, dehydration in a graded ethanol series (20 min at 30%→50→75%→95%) and overnight retention in 100% ethanol, critical point drying, mounting on a support stub and coating with gold-palladium. The prepared stubs were viewed on an ISI-60 scanning electron microscope. Extensive filamentous growth was observed on the outer layer of the rotating biological contactor biofilm, and a dense filamentous, small single cell bacterial growth was observed after enlarging the outer layer specimens. A mixture of approximately 0.5 x 3 μm straight and comma-shaped rods at the subsurface of the biofilm was found.

Weber et al. (1978) used SEM to observe the physical and structural characteristics of biological growth on activated carbon exposed to the coagulated-settled raw sewage in complete-mix batch reactors. The following procedure was used to prepare the samples: (1) immerse sample for 2 h in 2% paraformaldehyde-2.5% glutaraldehyde solution in 0.1 M sodium phosphate buffer at pH 7.3, (2) postfix sample for 3 h by using 1% OsO_4 in 0.1 M sodium phosphate buffer at pH 7.3, (3) dehydrate sample by rinsing the particles in graded ethanol (50, 70, 80, 90 and 100%), (4) perform critical point drying in a 1:1 100% ethanol-amylacetate solution, (5) store overnight in amylacetate, (6) mount on aluminum stubs and coat with 100 Å gold in a slow-discharge coater to minimize charging and increase the conductivity of the biological material. They found that the predominant organisms were probably stalked protozoans. The attachment site for the stalk of the protozoan could be seen and appeared to be a sturdy, gelatinous structure.

Hayat (1975) found that compared to freeze drying, critical point drying has several disadvantages: (1) the specimen is exposed to considerable pressure and,

depending upon the gas used, a temperature of –40°C, (2) after drying, the specimen has to be taken out of the pressure chamber and transferred to a vacuum device equipped with the evaporation source. Little is known with regard to what happens to the specimen during its transfer from the pressure chamber to the vacuum chamber.

Dempsey (1981) used SEM with freeze drying to observe bacterial biofouling of plastic and glass overslips. Standard methods of fixation and drying were used to prepare material for the SEM. Fixation was in 4% (v:v) glutaraldehyde, made up in either seawater or a 0.25 M sucrose (iso-tonic with seawater) -0.1 M cacodylate buffer (pH 7.2) mixture, for a minimum of 1 h. To minimize shrinkage and distortion artifacts caused by drying, freeze drying and critical-point drying were used in placed of air-drying. No appreciable differences were observed between the two methods, and for this reason, freeze-drying was routinely used for the majority of specimens. For freeze-drying, fixed specimens were rinsed for 5 min with double-distilled water by placing under the outlet of a still. Excess water was removed and the specimens were frozen by plunging into a liquid nitrogen slush (-210°C). They were then transferred, under liquid nitrogen, to the pre-cooled chamber of a freeze-dryer which was evacuated once all the liquid nitrogen had boiled off. Specimens were left to dry until a steady vacuum reading of less than 10^{-1} torr was achieved. Once dry, they were allowed to warm to room temperature by immediate transfer to a vacuum desiccator containing silica gel. They found that adsorption of non-living material led to the development of a conditioning layer. A range of stalked bacteria commonly is the secondary periphytes in microfouling communities, and permanent adhesion is achieved when mucilage is secreted by the bacteria. Bacterial colonies become embedded in confluent films of extracellular polymeric material.

Environmental scanning electron microscopy (ESEM) is a new development in SEM technology, where wet biofilms can be observed directly. Little et al. (1991) compared images of biofilms on metal surfaces obtained with ESEM with those from

SEM. Biofilm preparation for SEM followed this method: biofilms on metal surfaces were fixed in 4% glutaraldehyde buffered with sodium cacodylate (0.1 m, pH 7.2) at 4°C for a minimum of 4 h, washes with distilled water, rinsed through a series of distilled water/ethanol washed to ethanol, rinsed with amylacetate and critical-point dried from liquid CO_2. Dried specimens were sputter-coated with gold. The procedure of preparing the biofilm for ESEM was listed below: biofilms on metal surfaces were fixed in 4% glutaraldehyde in filtered seawater or cacodylate buffer (the same as for SEM preparation), rinsed to distilled water and examined in the ESEM. The authors found that ESEM images of biofilms indicated a gelatinous layer, with bacteria and microalgae enmeshed in a gelatinous film. However using SEM, much of the extracellular polymeric material from the biofilm was removed or dried to a thin inconspicuous residue during sample preparation.

Cunningham et al. (1991) used image analysis to measure the thickness of biofilm in the laboratory. A 10 power microscope lens was installed to observe biofilm attached to porous media, black and white photographs taken through the microscope lens were subsequently studied by image analysis. It is almost impossible to adapt this method at the pilot plant because a microscope lens has to be installed and moved along the filter to measure the thickness along the depth of the filter. Also, the magnification of microscope lens is too low to observe biofilm which may be only be a few μm thick.

Lee and Fuhrman (1987) used epifluroscence microscopy combined with acridine orange staining (ADOC) to estimate the volume of bacteria found in soil. Bacteria were filtered through a 0.6 μm filter and then filter was stained with acridine orange. Stained bacteria were observed under the microscope. Cells were categorized as rods when the length of two perpendicular axes (the longest and the shortest) differed by ≥0.1 μm; otherwise they were classified as spheres. Since cells of "rod" shape are not always true rod forms with two spherical ends on a cylinder, nor oblate spheroids with circular cross-

sections, volumes were calculated based on the two shapes, a rod and a spheroid. Biovolume estimation by this method needs a very large number of cells, partly because of the resolution of the photographs as well as because of visual problems in differentiating cell size to 0.1 μm. 200 to 250 cells per photographic slide were counted. This method underestimates the volume of the biofilm by only estimating the volume of bacteria without considering the extracellular polymeric substances (EPS). Therefore, it can not be adapted to estimate the thickness of biofilm although thickness can be estimated from the volume of the biofilm if the surface area of the support media is available (the thickness equals the biovolume divided by the surface area). Also, the method overestimates the volume of the viable biofilm since it can not distinguish viable bacteria from dead bacteria.

Cunningham et al. (1991) estimated the thickness of biofilm based on media porosity (α). Biofilm thickness L_f was calculated from observations of α at various stages of biofilm development. If biofilm is assumed uniformly distributed over media particles, an estimate of biofilm thickness can be made as following:

$$L_f = V_f/S \qquad 1$$

where V_f is the biofilm volume, determined as the difference between V_p values measured before and after biofilm accumulation, V_p is the pore volume in media sample, which can be measured by using the equation: $\alpha=V_p/V_t$ (V_t: total volume of media sample), and S is the total surface area inside the porous media reactor.

$$\alpha=v/v_p \qquad 2$$

where α is the effective porosity, v is specific discharge (flow rate/cross-sectional area) for the reactor, v_p is the average linear pore velocity through the porous media. Measurements of v were determined volumetrically, while corresponding measurements of v_p were made using bromothymol blue dye tracer monitored with a pair of infrared light

sensors mounted at either end of the reactor. This method can only be used to measure the thickness of biofilm attached to sand and anthracite because the pores in GAC may adsorb bromothymol blue dye tracer.

Rittmann et al. (1986) estimated the thickness of biofilm attached to glass beads by measuring weight difference before and after drying in a 103°C oven. Since water comprises about 99% of a biofilm's mass, the thickness of the biofilm could be estimated from the water content and the surface area of the glass beads:

$$L_f = W/(\rho n A\ (0.99)) \qquad 3$$

W is the weight of evaporated water (M), ρ is the density of water at 23°C (M/L^3), n is the number of glass beads, and A is the surface area per bead (L^2).

GAC particles contain pores which can be filled with water, and at least some of this water will be evaporated from the pores when dried at 103°C. W will therefore be much higher than its actual value. Since anthracite and sand do not have that problem, this method should be able to be used directly for anthracite and sand.

3.2 Methods for Measuring the Density of Biofilm

To measure the density of the biofilm, biomass has to be measured first, so the density can be estimated from the biomass and thickness of the biofilm (density equals the biomass divided by the volume of the biofilm, which equals the thickness multiplied by the surface area of biofilm). The method developed by Rittmann et al. (1986) is a typical example of this approach.

Rittmann et al. (1986) estimated density of biofilm attached to glass beads by measuring the COD (chemical oxygen demand) of the biomass.

$$X_f = (\text{COD of biomass (mg)} \times 0.706 \text{ mg biomass/mg COD})/nAL_f \qquad 4$$

where, X_f is the density of the biofilm attached to the glass beads, n is the number of glass beads, L_f is the thickness of biofilm, and A is the surface area of a glass beads (L^2), the 0.706 factor assumes that the biomass can be represented by $C_5H_7O_2N$, the empirical formula for aerobic cells.

This method would not be able to measure the biomass of biofilm attached to GAC particles because GAC adsorbs organic matter. Accurate accounting for the amount of organics adsorbed is often not possible, and those organics could be released when measuring COD (chemical oxygen demand). Since anthracite and sand do not have this problem, this method can be used directly for anthracite and sand.

Different methods have been developed to measure biomass based on volatile suspended solids (VSS), and specific cellular materials such as ATP, protein, carbohydrates or organic nitrogen.

Standard VSS determination (APHA-AWWA-WPCF, 1990) could be used to measure the biomass of biofilm. Sample were weighed in a dish, placed in an oven at 103 to 105^{o}C overnight, ignited in a furnace at 550 $\pm$50^{o}C for 1h, transferred to a desiccator for cooling in a drying atmosphere, and weighed again. The difference between the first weight and the second weight is the VSS. The determination offers a rough approximation of the amount of organic matter present. In the case of GAC, this method is complicated by the tendency of the carbon to adsorb significant quantities of organic from the medium. Accurate accounting for the amount of organic adsorbed is often not possible. The use of the standard VSS procedure is further aggravated by weight changes caused by oxidation of GAC during the high temperature (550^{o}C) firing of the samples (Craik, 1990).

Organic nitrogen analyzed colorimetrically after a Kjeldahi digestion technique (APHA-AWWA-WPCF, 1985, section 420) has been used to measure the biomass of

biofilm in an anaerobic culture (Kindzierski, 1989). Samples were block digested with sulfuric acid, potassium sulfate, and mercuric sulfate (a catalyst) to convert organic nitrogen to ammonium sulfate. Alkaline phenol and hypochlorite were then reacted with the ammonia (ammonium sulfate) to form an indophenol blue color that was proportional to the ammonia concentration. A Technicon Autoanalyer was used to measure the sample color intensity at a wavelength of 630 nm. Free ammonia and ammonium nitrogen were analyzed prior to the Kjeldahl digestion. The difference between Kjeldahl-nitrogen and ammonia-nitrogen represented organic nitrogen.

Carbohydrate analyzed using a modification of the colorimetric procedure described by Dubois et al. (1956) has been used to measure the biomass of biofilm involving in an anaerobic culture. (Kindzierski, 1989). Carbohydrates react with phenol and concentrated sulfuric acid to form an orange color proportional to the concentration. A sample aliquot and make-up distilled water (total 10 mL) were added to a 100 mL round bottom boiling flask along with 10 mL of a 6% phenol solution. Then 50 mL of concentrated sulfuric acid was rapidly added by directing the stream of the acid onto the surface of the liquid contents in the flask. The flask was swirled for 2 min after which 3 - 5 mL samples of the hot solution were transferred to disposable glass cuvettes. The cuvettes were placed in the dark for 45 min to allow the solution to cool to room temperature. Standards, derived from dilutions of a stock glucose solution (freshly prepared at 1,000 mg/L), and a distilled water blank were also carried through the acid digestion procedure. A Bausch and Lomb Spectronic 21D spectrophotometer was used to measure color intensity of the samples and standards at a wavelength of 485 nm.

Liu et al. (1992) modified the carbohydrate method by scanning in a spectrophotometer from 650 to 350 nm for the absorbance maximum against a reagent blank. Lau and Liu (1993) used the modified method to measure the biomass of biofilm attached on the surface of glass plates placed at the bottom of a river. They found that the

biofilm biomass accumulation was substantially reduced as flow shear stress increased and that the maximum accumulation occurred under very low flow conditions.

Protein analyzed by using a dye-binding technique based on the differential color change in response to various concentrations of protein (Bradford, 1976, cited from Kindzierski, 1989) has been used to measure the biomass of biofilm involving in an anaerobic culture (Kindzierski, 1989). Samples were extracted with sodium dodecyl sulfate (SDS) to free the cell protein and assayed using the dye-binding technique. A sample was extracted with 1% v/v SDS for 15 min. The sample-SDS solution was added to a test tube and placed in a sonicator. The test tube was then transferred to a table top centrifuge and spun at 3,500 rpm for 10 min to settle particulate and cell debris. The sample was removed from the test tube and diluted 10-fold to give a final SDS concentration of 0.1%. An aliquot of the diluted solution (0.2 mL) was added to a disposable glass cuvette along with 5 mL of a dye-binding reagent. The cuvette was capped, inverted several times to mix, and placed in the dark for 15 min. Standards, derived from dilution of bovine plasma gamma gobulin and a distilled water blank, containing 0.1% SDS were reacted with the dye-binding reagent in a similar fashion. A Bausch and Lomb Spectronic 21D spectrophotometer was used to measure color intensity of the samples and standard at a wavelength of 595 nm.

Sonderhoff et al. (1992) measured the biomass of mammalian cell-hybridoma 2E11 by measuring the ATP content of cells. ATP was extracted from the cells contained in 0.2 mL of culture by adding an equal volume of extraction buffer, containing 5% trichloro-acetic acid (TCA) with 6 nmol/L EDTA, to the cell suspension, immediately after the samples were taken. The samples were vortexed rapidly for 5 s and incubated for 15 min. ATP concentrations were determined using a bioluminescence assay based on the generation of light as a by-product of the ATP-dependent conversion of luciferin to oxyluciferin by the enzyme luciferase. The extracted samples (50 μL) were diluted with

tris acetate buffer (0.1 mol/L tris with 2 mmol/L EDTA, pH 7.75) in order to fall within the linear range of the assay (10^{-11} to 10^{-6} mol/L ATP). A minimum 50 x dilution was used to avoid inhibition of the assay by TCA. The diluted samples (50 µL) were added to cuvettes containing 350 µL tris acetate buffer and 100 µL of monitoring reagent (luciferase, luciferin, and bovine serum albumin in tris acetate buffer). The luminescence was measured using an LKB Wallac 1250 Luminometer attached to a potentiometric chart recorder. Following each determination, 10-µl aliquots of known ATP concentration were added to the cuvettes to serve as internal standards for the determination of the ATP concentration.

All those standard techniques which are specific for cellular materials involve colormetric reactions and spectrophotometric adsorption measurements, and are therefore often useless in systems employing GAC. GAC tends to absorp the reagents dyes used and to interfere with the adsorption measurements (Craik, 1990). Kindzierski (1989) found that organic (Kjeldhal) nitrogen, carbohydrate and protein analyses were inadequate for the measurement of biomass quantities in anaerobic sludge even in the absence of GAC. Reproducibility was poor and low biomass concentrations in the medium were cited as the cause of the difficulty. Because the concentration of organic substances in drinking water is even lower than that present in anaerobic sludge, an even lower biomass is expected. Organic (Kjeldhal) nitrogen, carbohydrate and protein analyses are not adequate for the measurement of biomass quantities of the biofilm attached on the sand and anthracite.

Thermogravimetric analysis (TGA) is a modification of the standard VSS procedure (Craik et al., 1991). TGA gave a direct estimation of the proportion of attached biomass, as volatile solids on the GAC removed from the bioreactor. The problem of volatilization of the carbon was minimized by performing the analysis under a non-oxidative (N_2) atmosphere. Weight loss due to pre-adsorbed material and any

volatilization of the GAC that did occur was accounted for by incorporating into the calculations a reference TGA determination of an appropriate GAC control. A Dupont model 951 Thermogravimetric analyzer coupled to a Dupont model 1090 Thermal analysis system was used. The temperature program was as follows: heating at $50^{o}C\ min^{-1}$ from room temperature to $110^{o}C$ which was maintained for 15 min; subsequent heating at $50^{o}C\ min^{-1}$ to $550^{o}C$ which was held for 20 min. The actual final temperature was $\pm 5^{o}$ of the setpoint. Moisture adsorbed by the sample during transfer to the instrument was quickly removed at $110^{o}C$, thereby providing an initial dry weight. The amount of biomass on activated carbon, expressed as (g VS) (g activated carbon)$^{-1}$, was determined by TGA. The following equation was used to correct for the weight loss due to the pyrolysis of activated carbon and to adjust for the differences between the weight loss observed in nitrogen compared to the weight loss in air,

$$VS_{TGA} = B_{air} \cdot (V - A_{N_2})/(B_{N_2} - V) \qquad 5$$

where VS_{TGA} is the volatile solids determined by TGA at $550^{o}C$, g g^{-1}, B_{air} is the weight loss of biomass in air by TGA at $550^{o}C$, g g^{-1}, B_{N_2} is the weight loss of biomass in nitrogen by TGA at $550^{o}C$, g g^{-1}, V is the measured weight loss of the sample in nitrogen by TGA at $550^{o}C$, g g^{-1}, A_{N_2} is the weight loss of the control sample of activated carbon in nitrogen by TGA at $550^{o}C$, g g^{-1}.

This method may be inadequate for measuring biomass in water treatment, and it is further conflicted by the impossibility of getting an appropriate GAC control. Generally, filters filled with chlorine have been used as a control, but recently, research had found that there are still bacteria attached to such filters (Wende et al., 1989).

Servais et al. (1992) used a radio-labeled tracer method to measure the bacterial biomass and activity associated with GAC. In this method, the production of $^{14}CO_2$ by respiration of added ^{14}C-glucose at a saturating concentration was measured. The

experimental procedure is the following: 1 mL of ^{14}C-glucose solution (1-mM glucose containing 0.1-1 μCi) is added to a 2-mL GAC sample in a penicillin flask closed with a rubber septum. The 2-mL GAC sample consists of GAC and water in the same proportion as in the studied filters (about 75% w/w of water). After 3 hours' incubation at 20°C, the sample is acidified by adding 2 mL of 10% H_2SO_4 through the septum and then bubbled for 10 min to extract the CO_2, which is trapped in a mixture of Carbo-Sorb and Lipoluma (1/4 vol/vol). Radioactivity is determined by liquid scintillation with a Packard Tri-Carb scintillation counter. Then, this method was calibrated with suspensions of bacteria detached by the early backwashing procedure from an activated carbon filter before this measurement. Potential glucose respiration measurements and bacterial enumerations by the ADOC method (acridine orange staining) were performed on the same sample. The results show a good correlation (r=0.99) between the potential glucose respiration measurement and the ADOC method, with the correspondence factor being 1.1 mg C of bacterial biomass per nmole glucose respired per hour.

This method underestimates the amount biofilm by only measuring the activity of viable bacteria. By calibrating with the ADOC method which can not distinguish between dead and viable bacteria, this method overestimates the biomass of viable biofilm, also.

An electron transport system method has been used to measure activity within a biofilm (Blenkinsopp, 1989). All respiring microorganisms possess an active electron transport system. Electron transport system activity is a measure of the ability of microorganisms to reduce an indicator under defined conditions. Tetrazolium salts such as 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT) are commonly used as electron transport system indicators. INT is water-soluble in its oxidized form and, when reduced, forms a water insoluble deposit, iodonitrotetrazolium formazan (INT-formazan), within the cell. Extracted formazan can be measured spectrophotometrically to quantify electron transport system activity. There are currently two main

spectrophotometric methods used to measure electron transport system activity. The first, developed for marine plankton work, involves incubating an enzyme extract prepared from the cells with an INT solution and a substrate solution containing electron transport system stimulators (NADH, NADPH and succinate). This method gives the maximum potential electron transport system activity of the community. The second method, described for soil work, involves incubating the "intact" microbial community in an INT solution without electron transport system stimulators. This gives a lower value than the first method because it measures the *in situ* electron transport system activity. A standard curve between biomass and electron transport system activity is not available, therefore the biomass of the biofilm can not be interpreted from the electron transport system value.

In another approach to biomass measurement, Findlay et al. (1989) found that a variety of organophosphates could be recovered quantitatively in a complex matrix and at concentrations typical of organic-rich marine sediment. 5 nmol of phosphatidylethanolamine was added to an environmental lipid extract to determine the recovery efficiency of lipid in a natural sample matrix. The amount recovered, calculated as (environmental lipid + phosphatidylethanolamine mixture)- environmental lipid, was 4.81±0.09. The recovery efficiency, calculated as (recovered phosphate/expected phosphate) x 100, was 96.0%.

This method has been largely used for determining biomass in sediments by microbiologists. Using the same type of method, Federle (1983) was able to show a dramatic decrease in microbial biomass as a function of depth in three soils.

The method developed by Findlay et al. (1989) has been adapted to measure the biomass of biofilm in water treatment by Summers and co-workers at the University of Cincinnati (Miltner et al., 1993). Biomass was determined by measuring the phospholipid concentration of the biofilm. Phospholipids are contained in cell membranes and are

common to all cells, which are not stored but turned over relatively rapidly during metabolism. Consequently, they indicate the viable biomass. The total lipids can be extracted from biomass attached on the surface of filter media by chloroform-methanol-water mixture, digested with oxidant (potassium persulfate) to release phosphate and the released phosphate is complexed with ammonium molybdate and malachite green, and measured colorimetrically. The procedure is quantitative, fairly simple and sensitive and can detect about 10^8 bacteria the size of *Escherichia coli*.

Samples were analyzed by following this procedure: the collected samples were prewashed with distilled water to remove all flocculated particles, 0.5 g to 1.5 g media were added to a 20 ml vial immediately after prewash, chloroform, methanol and acidified water (0.144 M sulfuric acid) were added to the vial in a ratio 1:2:0.8 such that the volume of the extraction mixture in milliliters was about ten times of the weight of the media in grams. The extraction mixture was then allowed to stand for 2 to 24 h and then separated into a lipid-containing chloroform phase (lower) and methanol-water aqueous phase (upper) by the addition of chloroform and acidified water such that the final ratio of chloroform-methanol-water was 1:1:0.9. A portion of chloroform was transferred to 10 mL ampoules and dried down under nitrogen gas, 0.9 ml potassium persulfate was added in ampoules immediately after the chloroform had been dried down, and the ampoules were sealed and heated at 100°C for 2 to 24 h. The ampoules were opened, 0.2 mL ammonium molybdate was added and allowed to stand for 10 minutes, 0.9 mL malachite green was added and allowed to stand for 30 min, and then the absorbance of the sample was measured by spectrophotometer.

The biomass of the biofilm attached on different drinking water filter media of a pilot plant were measured by Wang and Summers (1993) using this method, and the result was found to be very reproducible.

A conversion factor between the amount of phospholipid and the number of bacteria in a biofilm has been found by Balkwill et al. (1988). They measured four different components of phospholipid: phospholipid fatty acids, lipid phosphate, glycerol phosphate and LPS-OHFAME (lipopolysaccharide lipid A-hydroxy fatty acid methyl esters). The conversion factors for each component were respectively 100, 50, 50, and 15 μmol/g dry wt cells (1 g dry wt cells = 2 x 10^{13} cells). The measurements of four different phospholipid components gave essentially identical estimates of cell numbers and dry weight as the direct counts of the biomass in soil/sediment.

3.3 Methods Selected for Initial Evaluation

Based on the literature review, different methods are suitable to measure the thickness and the biomass of biofilm attached on different media such as sand, anthracite or GAC. But methods which are able to measure the thickness and the biomass attached to all of the media are needed to reduce the systematic error in comparing the thickness and density of the biofilm attached to different media. Therefore, the following methods were selected for initial evaluation with respect to measuring the thickness of biofilm: 1. SEM combined with critical point drying and freeze drying (if SEM is not suitable, ESEM will be evaluated), 2. epifluroscence microscopy combined with the ADOC method, 3. measuring the moisture loss on drying in a 103^{o}C oven. The phospholipid determination using malachite green was selected for evaluation of the biomass of biofilm.

Chapter 4 INITIAL SCREENING OF METHODS

To find a suitable method to measure the thickness and the biomass of the biofilm, the efficiency of each of the selected methods mentioned in Section 3.3 was evaluated using samples obtained in the laboratory or from a pilot plant to be discussed in Section 5.1.

4.1 SEM with Critical Point Drying and SEM with Freeze Drying

4.1.1 Materials and Methods

4.1.1.1 Samples

Two different glass bead samples were used: one is designated as Sample 1, the other as Sample 2.

The column from which Sample 1 was obtained was installed at the laboratory. Figure 1 illustrates the experimental setup. The column is 2.5 cm x 10.0 cm and the volume is 50 mL. It contains about 2600 3 mm glass beads, which provide a specific area of 1469.5 m^{-1}. The feed rate (Q) is about 1-2 ml/min and the recycle rate (Q_r) is about 50 mL/min. With the ratio of Q_r/Q greater than 25, the biofilm column can be regarded as a CSTR (Rittmann et al., 1986). The total flow rate of $Q+Q_r$ results in an approach velocity of about 7 m/h, which is in the mid range of hydraulic loading rates used in the pilot study. The feed is synthetic water, containing 25% acetate, 30% oxalate, 20% glyoxylate and 25% acetaldehyde, based on their theoretical oxygen demand (ThOD). The feed concentration was initially 10,000 µg/L acetate C equivalents on a ThOD basis, but was later reduced to 7,500 µg/L.

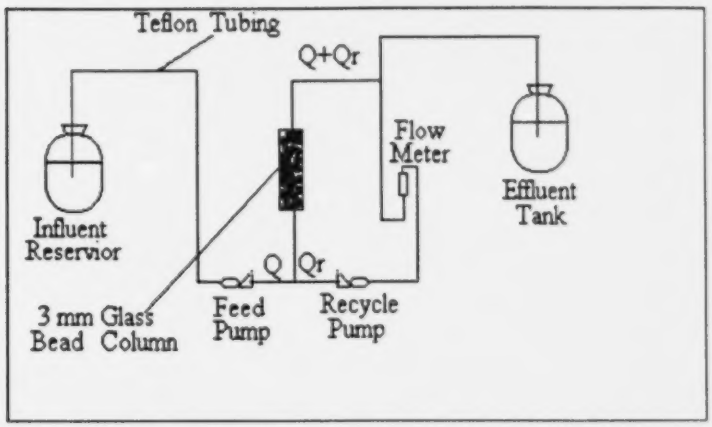


Figure 1 Schematic of Glass Beads Column Installed in the Laboratory

The column from which Sample 2 was obtained was installed at the pilot plant. Figure 2 illustrates the experimental setup. This column is 50 ml. It is tightly packed with 3 mm glass beads as attachment media. A pump feeds ozonated water to the column at a constant rate of 50 L/day. The column is operated in an upflow mode.

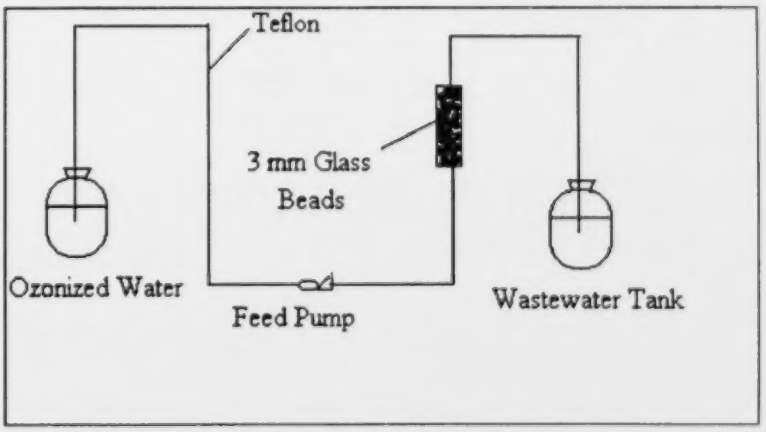


Figure 2 Schematic of Glass Beads Column Installed at the Pilot Plant

4.1.1.2 Sample Collection

Ten beads were taken one by one from the bottom of each of the two columns by using an incubating loop and were added into a 20 mL vial contained 2.5%

glutaraldehyde. The columns had been operating for several weeks prior to sample collection.

4.1.1.3 Description of Methods

Sample 1 was observed by SEM (HITACHI S-2500). The Sample was prepared by following the following procedure for SEM (critical point drying): prefix in 2.5% glutaraldehyde in Millonig's buffer or cacodylate buffer (pH 7.2) at reaction time for 1 hour, wash in the same buffer for 3 X, 10 minutes each, postfix in 1% O_sO_4 in the buffer using a reaction time for 1 hour, wash in distilled water briefly, dehydrate in a series of ethanol solutions (50%, 70%, 80%, 90%, 100%), 10 minutes each followed by two additions of absolute ethanol, 10 minutes each, critical point dry at 31°C for 5-10 min, mount on the stub and dry overnight in a vacuum desiccator, sputter coat (Edwards, Model S150B Sputter Coater) with gold.

Sample 1 and Sample 2 were observed by using SEM (Cambridge Stereoscan). The samples were prepared by following the procedure for SEM (freeze drying): fix 2-3 h in 3% glutaraldehyde in phosphate buffer at 4°C, wash 2 h (4 changes at 30 min intervals) in phosphate buffer with 10% sucrose at 4°C, postfix in 2% OsO_4 in phosphate buffer for 1-1/2 to 2 h at 4°C, dehydrate in 30% ethanol at 4°C approximately 15 min, freeze dry in a freeze-dryer (Edwards Pearse tissue dryer) overnight, and sputter coat (Natotech SEMPREP 2 Sputter Coater) with gold.

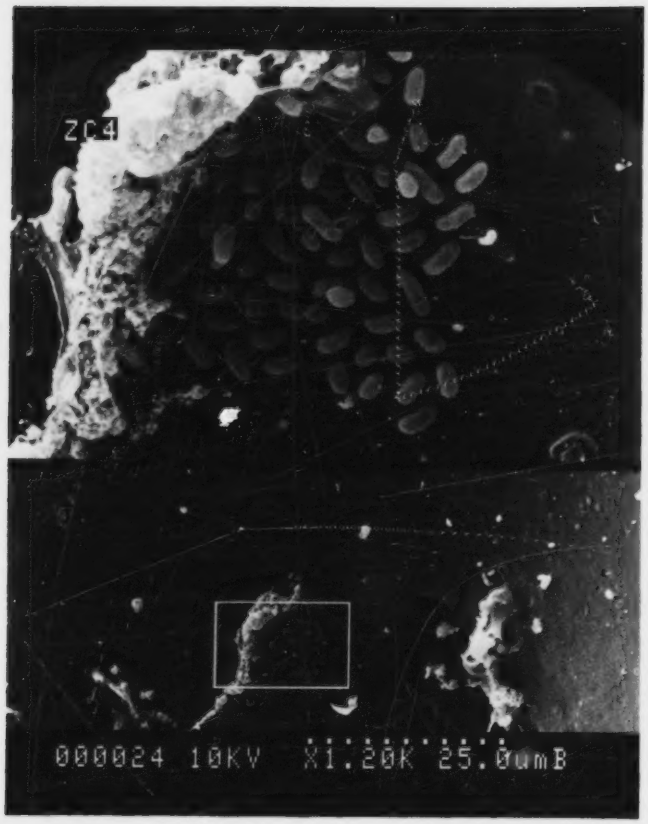
4.1.2 Results

Figure 3 is a selection of various SEM (critical point drying) photographs of Sample 1. In pictures A and B, isolated bacteria were observed on the surface of the glass beads, however no EPS (extracellular polymeric substance) and no biofilm were observed.

In pictures C and D and which are higher magnification photographs of photos A and B, a second layer of bacteria was observed, although no EPS and no biofilm were noted.

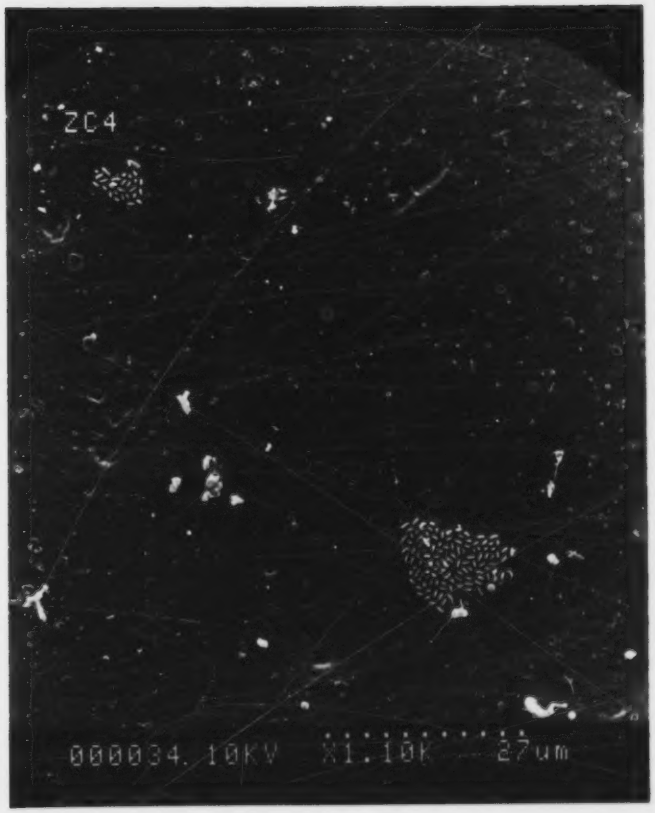
Figure 4 is a selection of various SEM (freezing drying) photographs of Sample 1. In photograph A, isolated bacteria and EPS were observed; in photograph B, EPS was observed; and a layer of film was observed by comparing photograph D (the surface of the sample) to photograph C (the surface of the blank - a brand new glass bead).

Figure 5 is a selection of SEM (freeze drying) photographs of Sample 2. In photographs A and B, isolated bacteria and EPS were observed, and patchy biofilm was noted.

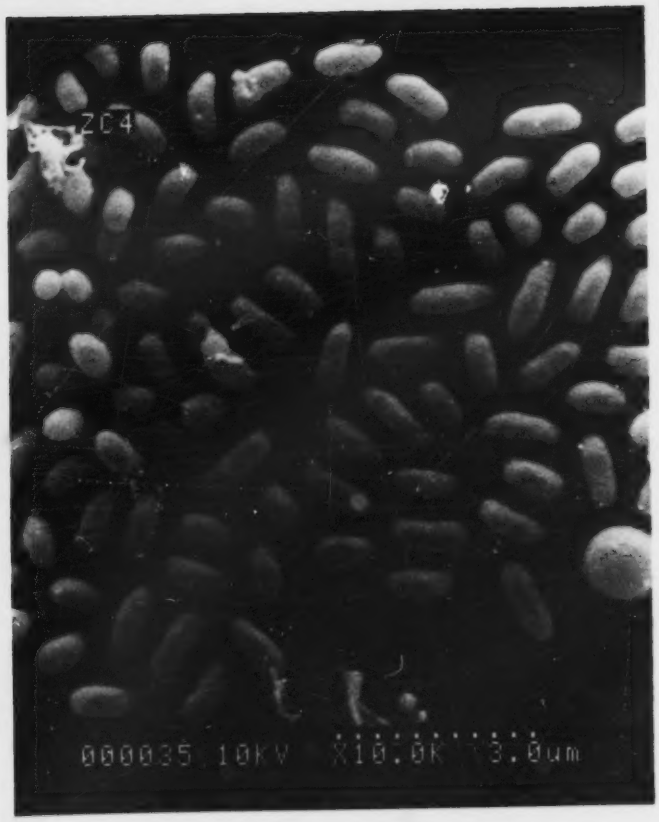


B

A



C



D

Figure 3 **A selection of various SEM (CPD) photographs of Sample 1. A: isolated bacteria group on the surface of glass bead; B: the high magnification of A; C: two isolated bacteria groups on the surface of glass bead; D: the high magnification of the bacteria group on the right corner of C.**

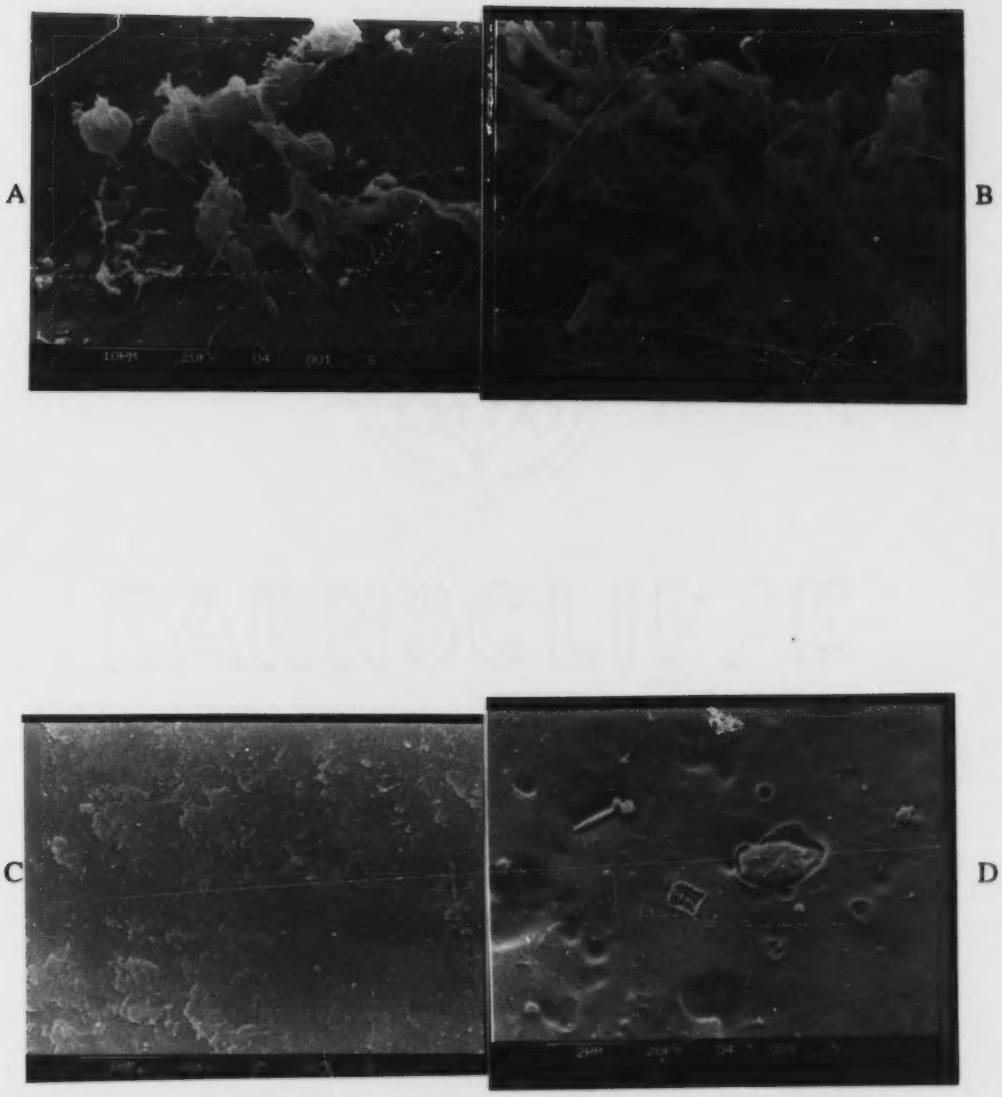
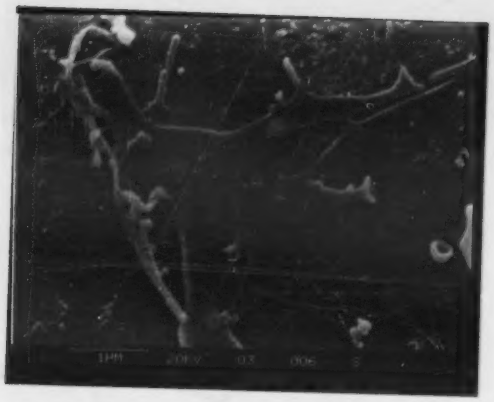


Figure 4 **A selection of various SEM (freeze drying) photographs of Sample 1. A: EPS on the surface of glass bead; B: EPS on the surface of glass bead; C: the surface of a blank glass bead; E: the surface of the sample glass bead.**



A

B

Figure 5 **A selection of various SEM (freeze drying) photographs of Sample 2. A and B: patchy biofilm on the surface of glass bead.**

4.1.3 Discussion

Before Sample 1 was sent to the SEM lab, the beads were observed with the naked eye. An unknown film was found to cover the beads completely, and even after the beads were dried at 103°C for 4 h, the same film still covered the beads completely. Regardless of whether it was biofilm or just a film of inorganic and/or organic substances, a film or deposit should have been observed by SEM. However, only a few isolated bacteria were seen using SEM (critical point drying).

More bacteria and EPS were observed by SEM (freeze drying) than SEM (critical point drying) for Sample 1, the sample examined by both techniques. A layer of film were observed by SEM (freeze drying). SEM (critical point drying) definitely causes shrinkage of the biofilm.

Under water treatment conditions and in the winter season, a thin, patchy biofilm would be expected. Patchy biofilm was found by Bouillot et al. (1992), who used scanning electron microscopy to observe the biofilm attached to filter media in a water treatment plant located in the suburbs of Paris, France. They found that sand granules were mainly covered by a mineral deposit, and a heterogeneous bacterial community entrapped in a network of filamentous species was only observed in cracks of the surface. In contrast, patches of a relatively abundant biofilm were present on the rough outer surface of GAC. Since the surface of glass beads is much smoother than the surface of sand, it is more difficult for bacteria to attach to it, and less biofilm would be expected, the pictures of Sample 2 are a vivid picture of the biofilm.

Since the SEM (freeze drying) provided a reasonably accurate image of the biofilm, it was considered not worthwhile to use the ESEM since the sample would have had to be sent to the United States, and the cost of analysis would have been high.

4.2 Estimation of Thickness by Measuring Moisture Loss

The method, developed by Rittmann et al. (1986), involves measuring the moisture loss at 103°C.

4.2.1 Materials and Method

Sample 1 was used to evaluate the efficiency of this method.

Ten beads were taken one by one from the bottom of the column of glass beads using an incubating loop to preclude inclusion of interstitial water. The beads were placed in a glass bottle which was covered by a rubber cap.

The beads with biofilm were weighed before drying in a 103°C oven, dried in a 103°C oven for 4 hours, cooled in a desiccator overnight, and then, weighed again. Triplicate analysis were done.

4.2.2 Results and Discussion

The results are listed in Table 1:

The thickness of the biofilm is calculated according to Equation 3:

$L_f = W/(\rho n A\ (0.99))$

in which W is the weight of evaporated water (M), ρ is the density of water at 23°C (M/L^3), n is the number of glass beads, and A is the surface area per bead (L^2).

$A = 4\pi r^2 = 4\ \pi\ (1.5\ mm)^2 = 28.26\ mm^2$

and $\rho = 0.997\ g/cm^3$

so, L_f= W/(ρnA (0.99))= 0.0068 g / (0.997 g/cm^3 x 10 x 28.26 mm^2 x 0.99)= 24 μm.

By using SEM (freeze drying), biofilm on beads obtained at the same time from Sample 1 was found to be very thin (about 1 μm) and patchy. Therefore, the Rittmann method seriously overestimated the thickness of the biofilm. This method was developed for estimating the thickness of a uniform and relatively thick biofilm. When the biofilm was thin and patchy, this method was not suitable to measure the thickness of the biofilm.

Table 1. Result of the method for estimating the thickness of biofilm by measuring the moisture loss on drying at 103°C

Bottle no.	39	92	55
No. of beads	10	10	10
W_s(g) (before. 103°C)	39.2831	38.8943	38.9206
W_s (g) (after. 103°C)	39.2763	38.8873	38.9140
W (g)	0.0068	0.007	0.0066
$W_{average}$ (g)	0.0068		
Sw	0.0002		

Ws is the weight of the beads, W is the weight difference between the weight of sample before and after drying in the 103°C oven, Waverage is the average of W, Sw is the standard deviation of W.

4.3 Epifluorescence Microscopy Combined with ADOC Method and 4', 6-diamidino-2-phenylindole (DAPI)

4.3.1 Materials and Method

Sample 2, as well as samples from filter 3 and GAC column 3 and 5 (designated as F3, C 3 and C5) at the pilot plant were used to evaluate the efficiency of this method.

Sample 2 was collected from the bottom of the column by using an inoculating loop. Samples were collected from sample ports of the filter and GAC contactors using a Teflon® tubing. Each sample was added to a 10 mL vial containing 5.0% (w/v) glutaraldehyde. Three samples were collected from Filter 3, two samples from GAC column 3 and GAC column 5. The samples were obtained from port 1, 2 and 3 of the filter (designated as F3-1, F3-2, F3-3), and from the 460 mm (1.5 ft) and 920 mm (3 ft) sample ports of the GAC columns (designated as C3, 1.5 ft; C3, 3 ft; C5, 1.5 ft; C5, 3 ft).

Samples were observed using epifluorescence microscopy (400 power lens) to estimate the percentage coverage of the biofilm. The percentage coverage was estimated by dividing the surface area of media into ten equal parts, identifying the biofilm in each part. The following procedure was used to prepare the samples: stain the sample with 0.1% (w/v) ADOC for 2 min, wash the sample with phosphate buffer (13.6 g/L), and then stain the sample with DAPI (1 μg/L) in the dark for at least 5 min. Then the average thickness of the biofilm equals the percentage coverage multiplied by 1μm. Because the thickness of the biofilm in drinking water treatment was very thin, almost just the thickness of 1-2 layer of bacteria, 1 μm was assumed to be the thickness of biofilm.

4.3.2 Results and Discussion

The results (Table 2) showed that, in winter, the percentage coverage was very low and with increasing depth, the percentage coverage decreased, as expected. Because

of the low concentration of organic substances at the pilot plant, the biofilm attached to filter media or GAC contactor media was patchy and the percentage coverage was low.

4.4 Determination of the Phospholipid Concentration Using Malachite Green

To evaluate the efficiency of this method, four columns installed at the laboratory for biofilm kinetics experiments were used. All columns were similar, but the concentration of feed solution to each was different. Column 4, receiving a feed concentration of 7500 µg/L, had been used to obtain Sample 1 discussed in previous sections.

Table 2. Result for epifluoresecence microscopy combined with AO and DAPI staining.

Sample	Percentage Coverage	Average (%)	Thickness ($x10^{-2}$ µm)
F3-1	10%, 5%, 10%	8	8
F3-2	An: 2.5%, S: 0%, 0%	0.8	0.8
F3-3	0%,0%,0%	0	0
C3, 1.5 ft	1%, 0.5%, 0.5%	0.7	0.7
C3, 3 ft	0.5%, 0.5%, 0.5%	0.5	0.5
C5, 1.5 ft	1%, 1%, 1%	1	1
C5, 3 ft	1%, 1%, 1%	1	1
Sample 1	4%, 4%, 4%	4	4

An: anthracite, S: sand.

4.4.1 Materials and Methods

For column 1, the feed concentration was 200 µg/L, for column 2, the feed concentration was 500 µg/L, and for column 3, the feed concentration was 2000 µg/L All concentration are expressed as acetate C equivalents on a ThOD basis.

From 0.5 g to 1.0 g beads were taken one by one from each of the bottom of columns using an inoculating loop, and the samples were added into a 20 mL vial. Duplicate analysis were done (designated as Column 1-1, Column 1-2)

Samples were analyzed by the following procedure: the collected samples were prewashed with distilled water to remove all flocculated particles; 0.5 g to 1.5 g media were added to a 20 mL vial immediately after prewash; chloroform, methanol and acidified water (0.144 M sulfuric acid) were added to the vial in a ratio 1:2:0.8 such that the volume of the extraction mixture in milliliters was about ten times the weight of the media in grams; the extraction mixture was allowed to stand for 2 h and then separated into a lipid-containing chloroform phase (lower) and methanol-water aqueous phase (upper) by the addition of chloroform and acidified water such that the final ratio of chloroform-methanol-water was 1:1:0.9. A portion of the chloroform was transferred to a 10 mL ampoule and dried down under nitrogen gas, 0.9 ml potassium persulfate reagent (5 g/100 mL 0.36 N sulfuric acid) was added to the ampoule immediately after the chloroform had dried down and the ampoule was sealed and heated at 100°C for 24 h. The ampoule was opened, 0.2 ml ammonium molybdate reagent (2.5% $(NH_4)MO_7O_{24}$-$4H_2O$ in 5.72 N sulfuric acid) was added and the solution was allowed to stand for 10 min, 0.9 ml malachite green reagent (dissolve 0.111% polyvinyl alcohol (PVA) in water at 80°C, cool, then add 0.011% malachite green) was added and the solution was allowed to stand for 30 min. The absorbance of the sample was measured by a spectrophotometer (Milton Roy Company Spectronic 601) at 610 nm.

A standard curve was prepared using: 0.05 M K_2PO_4 as a standard solution. A Standard curve was prepared as follows: 0, 50, 100, 150, 200, 250, 300 µl of a 0.1 µM K_2PO_4 solution were added in 10 mL ampoules and the volume was corrected to 1000 µ L by the addition of distilled water. Then, the standards were analyzed following the procedure discussed above.

4.4.2 Results and Discussion

Figure 6 is the standard curve. The result of the evaluation are listed in Table 3.

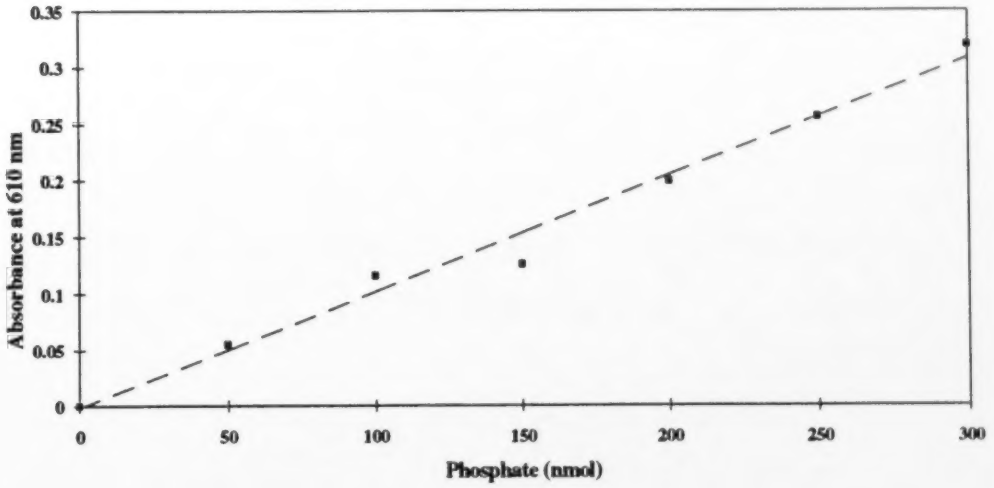


Figure 6. Standard Curve for determining phospholipid concentration using malachite green

Table 3. Result of the initial evaluation of the method for determining phospholipid concentration using malachite green

Sample	nmole P/g media	Average	Std. dev.
Column 1-1	6.57		
Column 1-2	6.07	6.32	0.36
Column 2-1	7.52		
Column 2-2	7.79	7.66	0.20
Column 3-1	9.91		
Column 3-2	9.64	9.77	0.19
Column 4-1	11.95		
Column 4-2	11.86	11.97	0.07

The results showed that the biomass increased from 6.32 to 11.97 nmol P/g media as the AOC feed concentration increasing from 300 to 7500 µg/L, but a non-linear relationship existed. This trend was as expected.

4.5 Summary

The SEM (freeze drying) method and the phospholipid determination using malachite green were selected for detailed investigation because the thickness estimated from SEM (freeze drying) photographs and the biomass measured by the phospholipid determination using malachite green provided respensentive values of thickness and biomass based on the initial evaluation results.

Chapter 5 MATERIALS AND METHODS FOR DETAILED INVESTIGATION

5.1 Description of the Pilot Plant

The description in this section is adapted from Mitton (1993)

A pilot plant was installed in the Rossdale Water Treatment Plant, in Edmonton, Alberta, raw water for the pilot plant was obtained from the North Saskatchewan River using the Rossdale intake. Figure 7 is a schematic of the pilot plant and Table 4 outlines the operating parameters. All materials in contact with water were made of stainless steel, glass or a fluorocarbon to prevent organic contamination.

Raw water flowed to the pilot plant at 40 L/min passing through a screen to remove stones and leaves. A presedimentation tank (not shown) provided protection from elevated turbidity levels and a blowdown flow of 10 percent of influent flow was maintained. Coagulation was achieved with polyaluminum chloride with a dosage ranging from 20 to 120 mg/L. Rapid mixing was accomplished with a variable speed agitator with a detention time of 17 seconds. A tapered three stage tapered flocculation followed with a detention time of 37 minutes. A velocity gradient was achieved with decreasing impeller speeds of 40, 20 and 12 rpm. Clarification was completed in a sedimentation tank containing of 60^{o} parallel plates spaced at 50 mm intervals. Following sedimentation a settled water storage tank served to provide a hydraulic buffer prior to downstream units, and to provide a nearly constant head for the pumps feeding those units.

A portion of the settled water was pumped into an ozone contactor operating in a countercurrent mode. The dose applied to the contactor was initially 1.0 mg/L ozone per mg/L NVOC, however the operating procedure was changed later in the study (March 11, 1993) to maintain a target ozone residual (0.1 mg/L) which lowered the applied dose to approximately 0.75 mg/L ozone per mg/L NVOC. The ozonated water was directed to a

small dissipation tank to allow a longer gas/water contact time. At this point the ozonated water was pumped to two anthracite-sand filters (Filters 2 and 3) and one GAC F-300-sand filter (Filters 4). Two other streams originated from the settled water storage tank. One stream, considered the control stream, was pumped to Filter 5 with no ozonation. The other stream was chlorinated to obtain a residual of 0.5 mg/L free chlorine prior to being pumped to Filter 1. Filtration was carried out in the constant-rate, variable-head mode. Filters 3 and 4 received a hydraulic loading of 5 m/h and Filters 1, 2 and 5 received a hydraulic loading of 10 m/h. Filters 1, 2, 3 and 5 contained 610 mm anthracite overlying 305 mm sand, and the filter adsorber (Filter 4) contained 610 mm GAC F-400 overlying 305 mm sand. The columns were backwashed manually every 24 hours using the collapse pulsing procedure developed by Amirtharajah et al. (1991). This involves a combination of air and water at sufluidization velocities in the first stage of the backwash followed by water alone to fluidize the bed in the second stage.

Filtered water was pumped to the GAC contactors, also operating at a constant rate, variable head mode. Contactors 2, 3 and 5 contained approximately 2.3 m of F-400 and contactor 4 contained approximately 2.3 m of Pica carbon. Contactors 3 and 4 received a hydraulic loading of 4 m/h and contactors 2 and 5 received a hydraulic loading of 8 m/h. The columns contained five sampling ports, representing contact times ranging from 1 to 17.5 min at the low loading rate and 1 to 34.5 min at the high loading rate. For each stream, the filters and GAC contactors were backwashed with stored effluent from that stream.

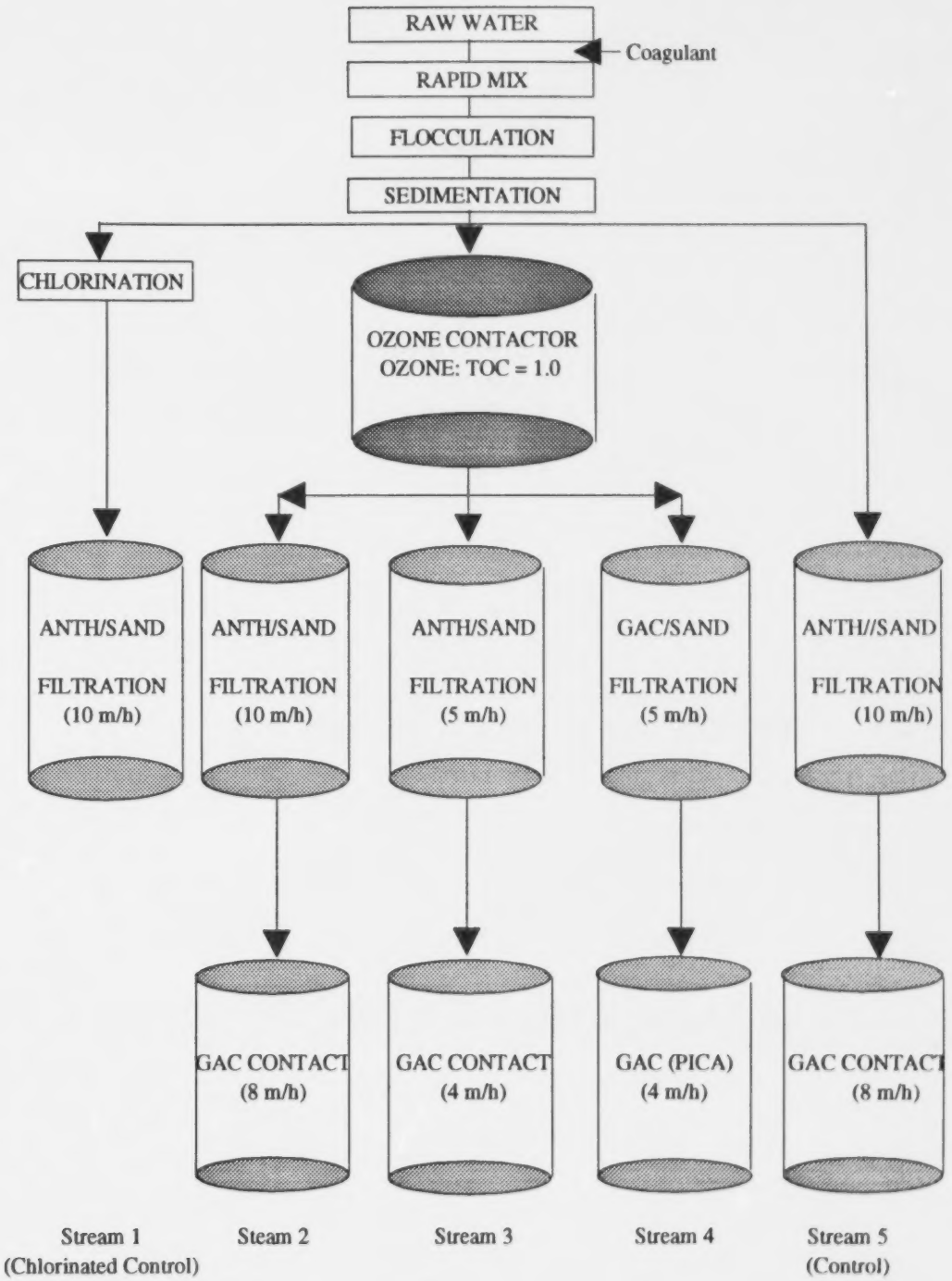


Figure 7: Schematic for the Edmonton, Alberta Pilot Plant 1992-1993 (Phase I) (Source: Mitton, 1993)

Table 4;
Operating parameters for Edmonton, Alberta pilot plant 1992-1993) (Source: Mitton, 1993)

Treatment Step	Parameter	Value
Raw water	Flow rate	40 L/min
Screening	Screen opening	3 mm
Presedimentation	Overflow rate	4.15 cm/min
	Detention time	9.3 min
Coagulation	Detention time	17 s
	Polyaluminum Chlorine Dosage	20-120 mg/L
Flocculation	Detention time	37 min (3 units total)
	Velocity gradient	40, 20, 12 rpm
Sedimentation	Parallel plates	60 ° inclination
	Plate separation	5 cm
	Detention time	69.8 min
Settled-water storage	Detention time	15.5 min
Ozonation	Detention time	
	Contactor 2	16.3 min
	Applied dose (target)	
	Contactor 2 to filters 2 & 4	1.0 ozone/NVOC
Filtration	Anthracite depth (Filter 1,2,3 &5)	610 mm
	Effective size	1.18 mm
	Sand depth (Filters 1,2,3,4, &5)	305 mm
	Effective size	0.45 mm
	GAC f-300 depth (Filter 4)	610 mm
	Effective size	0.75-0.85
	Hydraulic loading	
	Columns 3 and 4	5 m/h
	Columns 1,2 and 5	10 m/h
GAC columns	Carbon	
	Type (Column 2,3,&5)	GAC[1]
	Effective size	0.55-0.75 mm
	Sieve size	12-14 (US standard)
	Pore volume	0.94 cc/g
	Uniformity coefficient	1.9 (maximum)
	Particle density wetted-water	1.3-1.4 g/cc
	Total surface area (N_2 BET)	1050-1200 mm^2/g
	Bed depth	
	Column 2,3,4 and 5	2.8 m
	EBCT-Column 3 and 4	1-34.5 min
	EBCT-Column 2 and 5	1-17.3 min
	Hydraulic Loading	
	Column 3 and 4	4 m/h
	Column 2 and 5	8 m/h

[1] Filtrasorb 400, Calgon Corp., Pittsburgh, Pa

5.2 Sampling Procedure and Schedule

5.2.1 Media

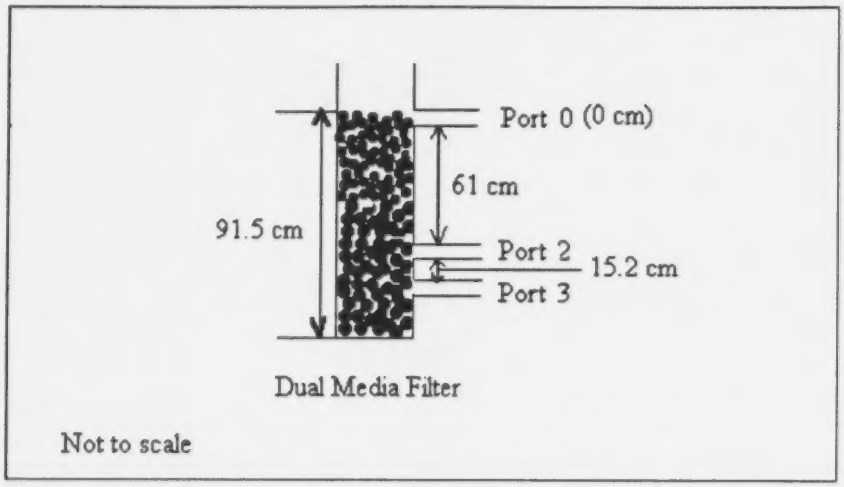
Samples were collected from sampling ports 0, 2 and 3 of the dual-media filters and filter-adsorber, sampling ports 0, 1 and 2 of GAC contactors 3 and 5 and sampling ports 0 and 2 of GAC contactors 2 and 4. From the top of the filters, port 0 is located at 0 cm, port 2 is located at 61 cm (the interface between the anthracite or GAC and sand), and port 3 is located at 76.2 cm. From the top of the GAC contactor, port 0 is located at 0 cm, port 1 is located at 138 cm, and port 2 is located at 218 cm. Figure 8 shows the location of the sampling ports. In practice, the samples for port 0 were obtained by inserting a long tube from the top of the filter or GAC contactor.

To collect samples from port 0 of the dual media filters, a 0.5 mm stainless steel tube was gently stuck into the filter from the upper floor. To collect samples from ports 2 and 3 of the dual media filters which are also liquid sampling ports, the sampling linets were removed (as had been provided for in the design) and a 1 mm stainless steel tube was gently stuck into the port.

To collect samples from port 0 of the GAC contactors, a 0.5 mm stainless steel tube was gently stuck into the contactor from the upper floor, as for the filters. To collect samples from sampling ports 1 and 2 of the GAC contactors, which are special sampling ports designed for collecting media samples, a scoop was stuck gently into the sampling port.

Samples for biomass measurement were put into small sampling bags and stored with the influent water of the filter or contactor from which they came. Samples for SEM examination were put into 20 mL vials containing 3% glutaraldahye.

Samples were collected from ports each week in the period from February 25, 1993 to March 24, 1993. During the spring runoff period from March 26 to April 1, 1993, samples were collected every three days. Each sample was designated with a specific symbol according to the sample source, Table 5 is the list of the symbols. Table 6 is the list of the samples collected on each sampling day.



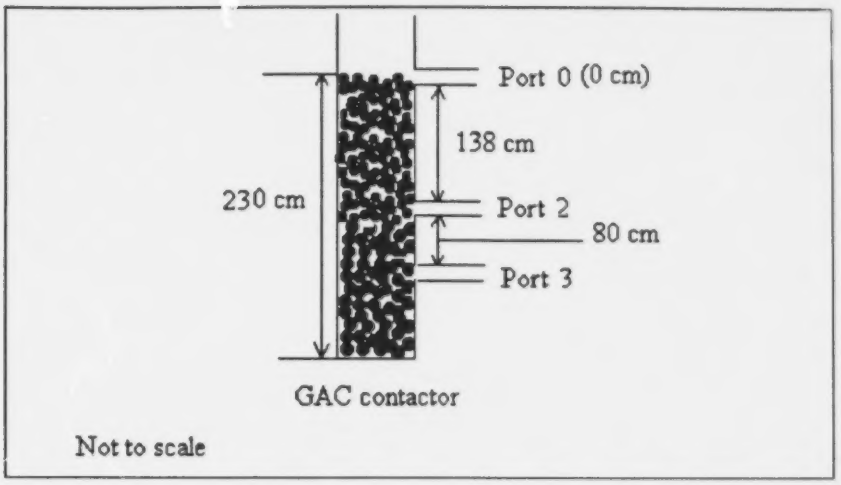


Figure 8 Location of Sampling Ports

5.2.2 Water Samples

Influent and effluent samples were collected from each of dual-media filters and GAC contactors for measuring the assimilable organic carbon (AOC) concentration and the heterotrophic plate count (HPC). Samples collection and analysis was performed by other members of the project team. Samples were collected routinely every two weeks in the period from February 1993 to March 24, 1993. During the spring runoff period from March 26 to April 1, 1993, samples were collected every three days. Each sample was designated with a specific symbol according to the sample source (Table 7).

5.3 Methods

For measuring the thickness of the biofilm, the SEM (freeze drying) method descried in Chapter 4 was followed. Thickness was estimated from the SEM photographs. If a uniform biofilm was presented, the thickness was estimated directly from the photo. If the biofilm was patchy, the percentage coverage and thickness of the biofilm were estimated, and then the effective average thickness was calculated by multiplying the percentage coverage by the thickness.

For each sample, at least three particles were observed by SEM, since there were variations among particles. To reduce costs, photographs were only taken of particles judged to have "average" biofilm, instead of all particles.

For measuring the biomass of the biofilm, the phospholipid determination using malachite green was used. For each sample, duplicate determinations were conducted.

Table 5 List of symbols used for media samples.

Symbol	Sample Source
F1-0	Port 0 of Filter 1
F1-2	Port 2 of Filter 1
F1-3	Port 3 of Filter 1
F2-0	Port 0 of Filter 2
F2-2	Port 2 of Filter 2
F2-3	Port 3 of Filter 2
F3-0	Port 0 of Filter 3
F3-2	Port 2 of Filter 3
F3-3	Port 3 of Filter 3
F4-0	Port 0 of Filter 4
F4-2	Port 2 of Filter 4
F4-3	Port 3 of Filter 4
F5-0	Port 0 of Filter 5
F5-2	Port 2 of Filter 5
F5-3	Port 3 of Filter 5
C2-0	Port 0 of GAC contactor 2
C2-2	Port 2 of GAC contactor 2
C3-0	Port 0 of GAC contactor 3
C3-1	Port 1 of GAC contactor 3
C3-2	Port 2 of GAC contactor 3
C4-0	Port 0 of GAC contactor 4
C4-2	Port 2 of GAC contactor 4
C5-0	Port 0 of GAC contactor 5
C5-1	Port 1 of GAC contactor 5
C5-2	Port 2 of GAC contactor 5

Table 6 The samples collected on each sampling day.

Sample	Date						
	Fab. 25	Mar. 3	Mar. 10	Mar. 24	Mar. 26	Mar. 29	Apr. 1
F1-0	x	x	x	x	x		x
F1-2	x	x	x	x	x		x
F1-3	x	x	x	x	x		x
F2-0	x	x	x	x	x	x	x
F2-2	x	x	x	x	x	x	x
F2-3	x	x	x	x	x	x	x
F3-0	x	x	x	x	x		
F3-2	x	x	x	x	x		
F3-3	x	x	x	x	x		
F4-0	x	x	x	x	x		x
F4-2	x	x	x	x	x		x
F4-3	x	x	x	x	x		x
F5-0	x	x	x	x	x		
F5-2	x	x	x	x	x		
F5-3	x	x	x	x	x		
C2-0	x	x	x	x	x	x	x
C2-2	x	x	x	x	x	x	x
C3-0	x	x	x	x	x		
C3-1	x	x	x	x	x		
C3-2	x	x	x	x	x		
C4-0	x	x	x	x	x		x
C4-2	x	x	x	x	x		x
C5-0	x	x	x	x	x		
C5-1	x	x	x	x	x		
C5-2	x	x	x	x	x		

Table 7 List of symbols used for water samples.

Symbol	Sample Source
R	Raw water
S	After sedimentation
CL	After chlorination
OZ	After ozonation
F1	Effluent of Filter 1
F2	Effluent of Filter 2
F3	Effluent of Filter 3
F4	Effluent of Filter 4
F5	Effluent of Filter 4
C2	Effluent of contactor 2
C3	Effluent of contactor 3
C4	Effluent of contactor 4
C5	Effluent of contactor 5

AOC measurements were conducted by technologist Janis Cook of the Department of Microbiology. The method of van der Kooij with minor modifications as descried by Huck et al. (1991) was followed. The first step of the procedure was the destruction of vegetative cells in the water sample to be tested by heating the sample to 60^{o}C in a water bath. Following this, the sample was then transferred to an oven for 30 min at 60^{o}C. After cooling, the sample was inoculated simultaneously with the precultured cells of P17 or NOX and incubated at 15^{o}C without shaking until the maximum number (Nmax) of colony forming units per milliliter (cfu/ml) was attained. Growth of the strains P17 and NOX was determined by periodic (usually daily) colony counts. Yield factors for P17 and NOX were obtained using acetate and agreed closely with values published by van der Kooij.

The HPC of the water was determined by The Provincial Laboratory of Public Health for Northern Alberta using the standard spread plate method. The procedure was listed as follows: select the dilution rate first so that the total number of colonies on a plate will be between 30 and 300. Then, pipet 0.1 to 0.5 mL diluted sample onto the surface of a predried R2A agar plate. Using a sterile bent glass bod, distribute the inoculum over the surface of the medium by rotating the dish by hand or on a turntable. Let the inoculum be absorbed completely into the medium before incubation. Incubate in a 20^{o}C incubator for 7 days. Count all colonies on selected plates promptly after incubation. Report count as CFU/mL by multiplying average number of colonies per plate by the reciprocal of the dilution used.

Chapter 6 RESULTS AND DISCUSSION

All biomass results are presented in the units in which they were obtained, i.e. nmol P/g media. All biomass were measured under wet sample basis, the variation in the amount of water with each sample would produce some variation. However, in some cases, biomass is also expressed as mmol P/m^3 reactor, because this is more meaningful from a process point of view. These results were obtained by multiplying nmol P/g media by the bulk density of the media. The bulk density of each media is listed in Table 8. Because the density of the Pica carbon was not available, it was assumed to be the same as GAC-F400. Since the percentage of anthracite and sand in the sample collected from port 2 of each dual media filter varied, these biomass values could not be converted to mmol P/m^3 reactor. The data analysis based on biomass expressed as mmol P/m^3 reactor for the dual media filters was therefore conducted without the interfacial data.

Table 8 The density of each media

Media	Bulk Density (g/cm^3)
Anthracite	0.83
Sand	1.46
GAC F300	0.44
GAC F400	0.43

The original biomass data are presented in Appendix B. A blank in any of the data tables indicated that no data were available for that sampling point.

When samples were collected, a tube or a scoop had to be stuck into the sampling port several times to get enough sample for analysis, so that a repesentive sample was

obtained. When samples were analyzed for biomass, duplicates were done for each sample on each sampling day. Generally, the duplicates matched each other very well except for samples collected from the media interface of the filters. Since the samples collected from the interface were not split into two equal parts, one sample could contain more sand or anthracite than the other. It was therefore reasonable to find some difference between the duplicates.

When the samples were analyzed for biomass, a blank control was obtained every time by following the same procedure. In general, a fairly good blank control (absorbance = 0) was obtained for each sampling day.

A control measurement of blank (unused) media was also conducted. Results showed that the biomass was 0 nmol P/g for each media.

The statistical procedures used to analyze the data consisted of paired t-test and linear regression analysis. In general, a one sided paired t-test was used unless otherwise specified. A value of $\alpha = 0.05$ was used to establish significance. Example statistical analysis are presented in Appendix D.

A conversion factor between nmol P/g media and the number of cell/g media was obtained from the literature. Biomass results have been converted into units of no. cell/g media in the overall discussion section.

6.1 Vertical Stratification of Biomass

6.1.1 Results Expressed As Unit nmol P/g media

Figure 9 shows the biomass as a function of depth in the dual-media filters and GAC contactors. Generally, the biomass of the biofilm decreased with depth, even if only slightly, in all filters and contactors except in Filter 1, the chlorined filter.

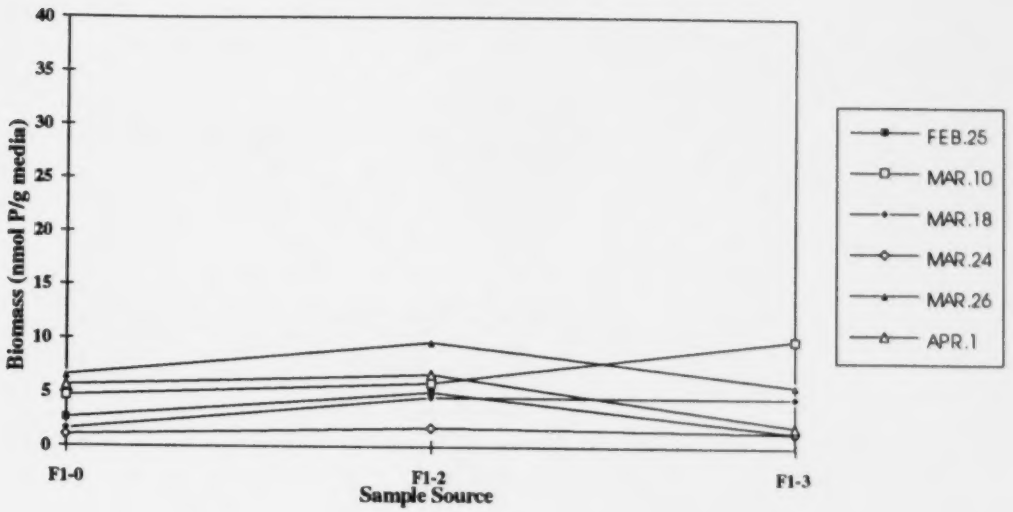


Figure 9 a. Filter 1.

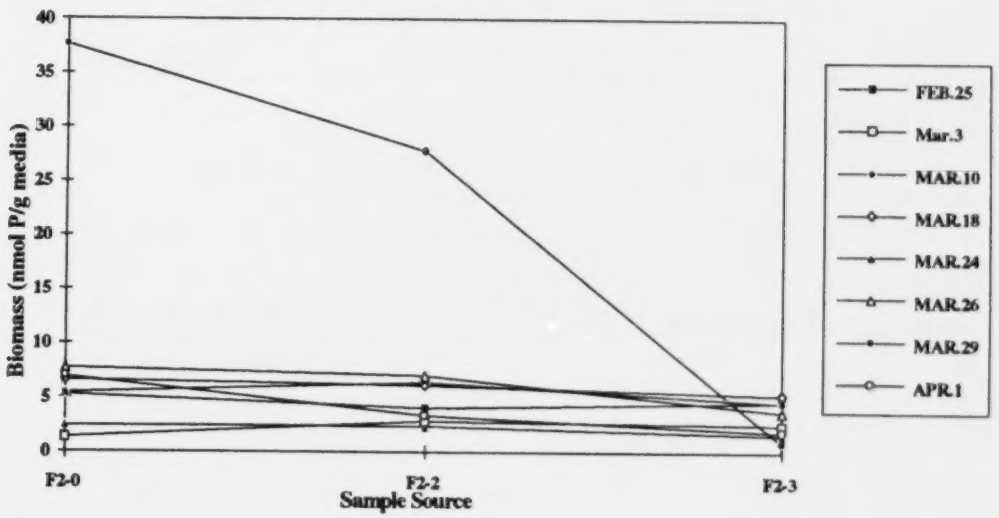


Figure 9 b. Filter 2.

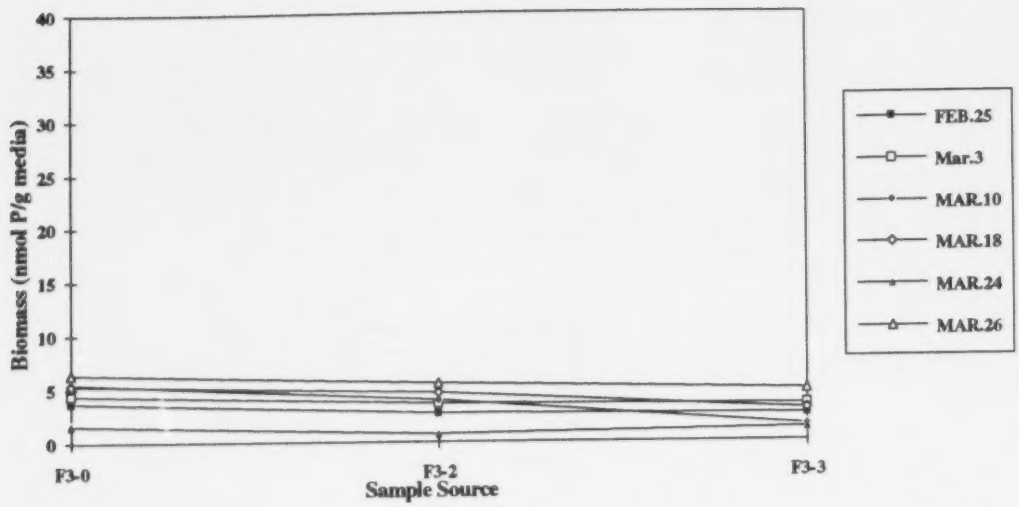


Figure 9 c. Filter 3.

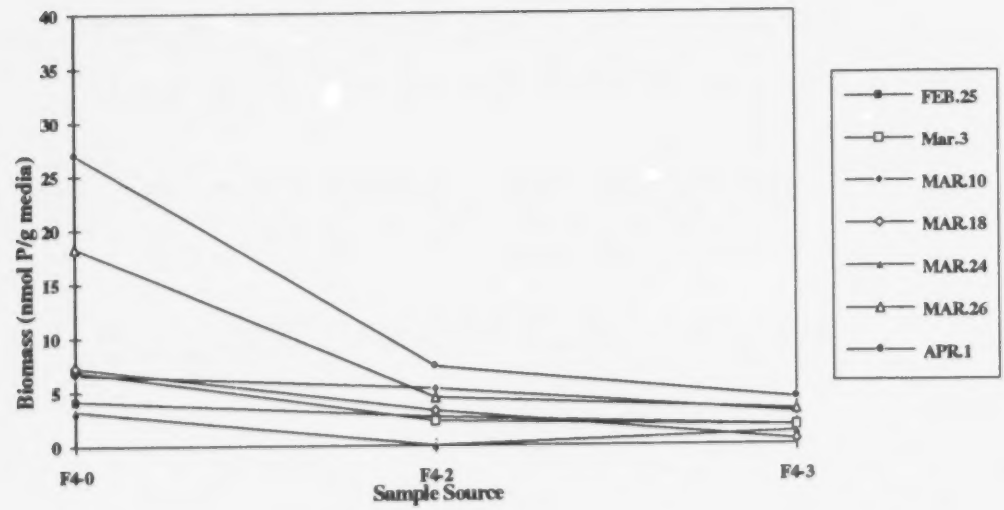


Figure 9 d. Filter 4.

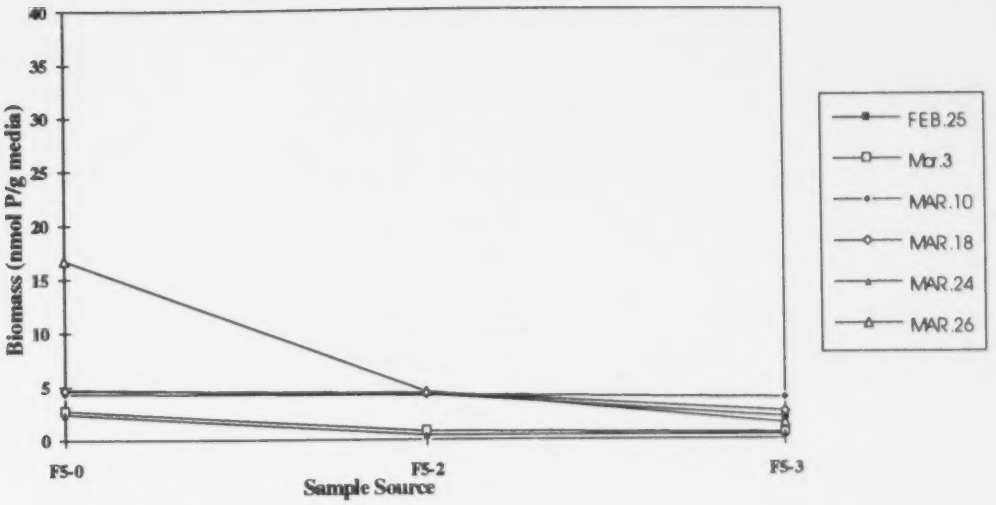


Figure 9 e. Filter 5.

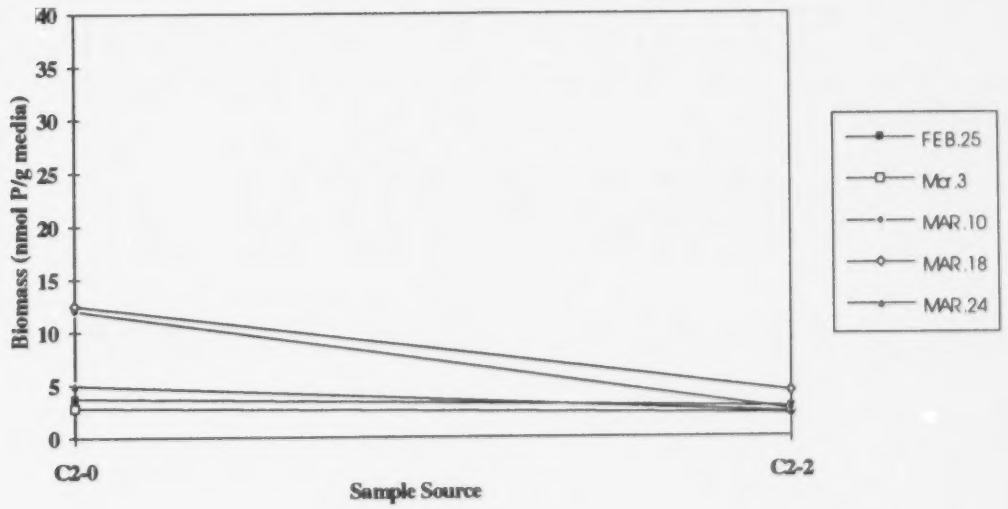


Figure 9 f. GAC2.

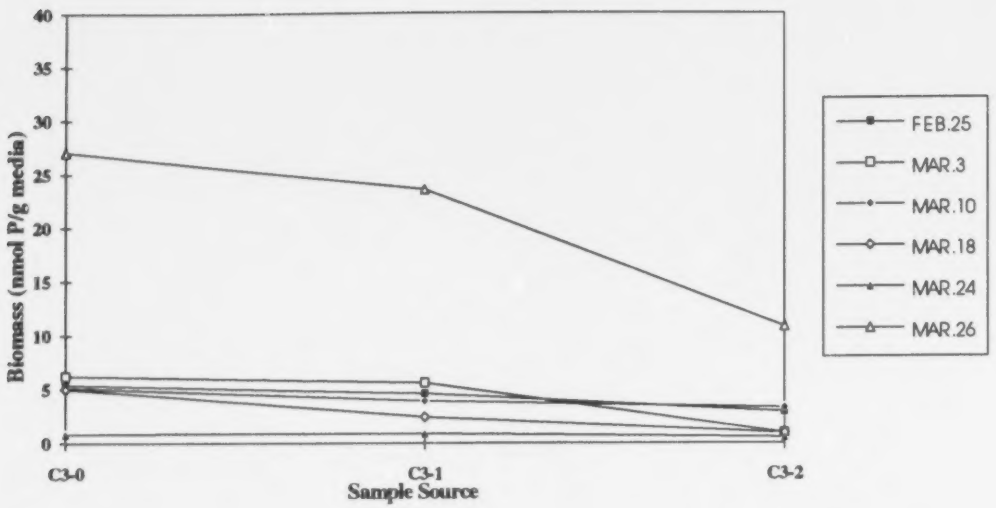


Figure 9 g. GAC3.

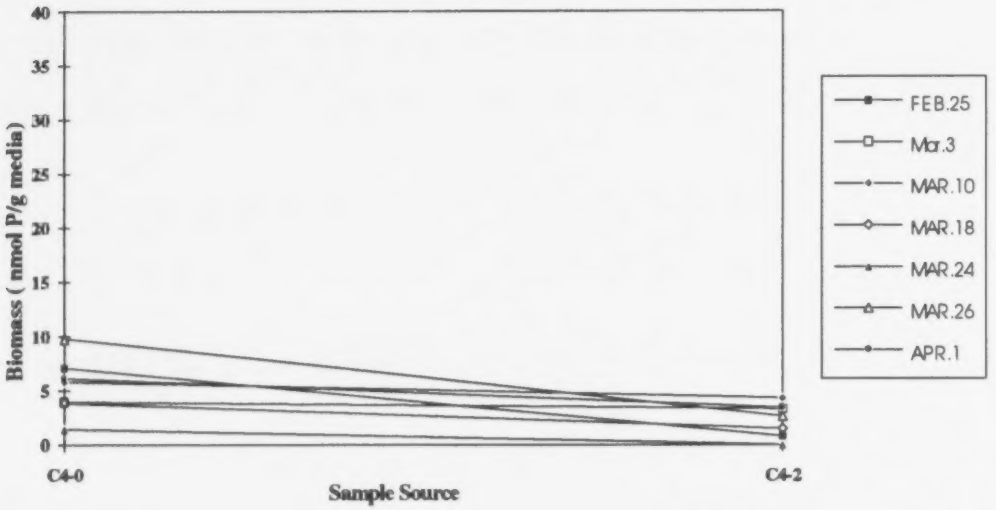


Figure 9 h. GAC4.

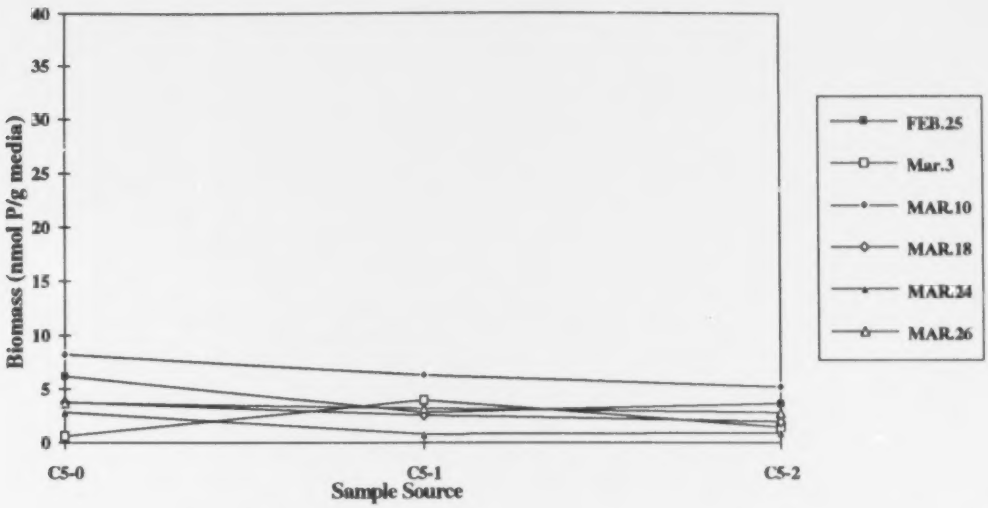


Figure 9 i. GAC5.

Figure 9 Vertical Stratification of Biomass Based on nmol P/g media.

In all filters and contactors except Filter 1, the biomass at the top of filter was the highest, even if by only a small amount. At the intermediate depth, it was generally less than at the top, and at the bottom part, the biomass was even less than at the middle port.

The biomass increased with depth in Filter 1, but two different trends were found. In the first, the biomass increased steadily with depth, as on March 24, 1993. In the second, the F1-2 value was the highest, and F1-3 was less than F1-0 (e.g. April 1).

High values of biomass have been observed in Filter 2 and GAC contactor 3 during spring runoff because of the relatively high concentration of AOC.

6.1.2 Results Expressed As mmol P/m^3 reactor

Different vertical stratification patterns in the filters were found when the biomass results were presented in mmol P/m^3 reactor. Figure 10 shows the vertical stratification

of each filter and contactor. Since the percentage of anthracite and sand in the sample collected from port 2 of each dual media filter (the port located at the interface between the anthracite or GAC and sand) was unknown, no results are presented for port 2, but line graphs will still be used in this section to facilitate comparison with the graphs in the last section. Also, because of the effect of the high ozone residual before March 18, which could produce a bactericidal effect in the filters, the data for the filters were divided into two groups to show the pattern clearly.

From February 25 to March 10, the biomass presented as mmol P/m^3 reactor, generally increased along the depth of each filter. From March 18 to April 1, the biomass generally decreased along the depth of the filter, except in Filter 3.

In the GAC contactors, the same vertical stratification patterns were found as for biomass present as nmol P/g media. The biomass decreased along the depth of the contactor except for GAC5 on March 3.

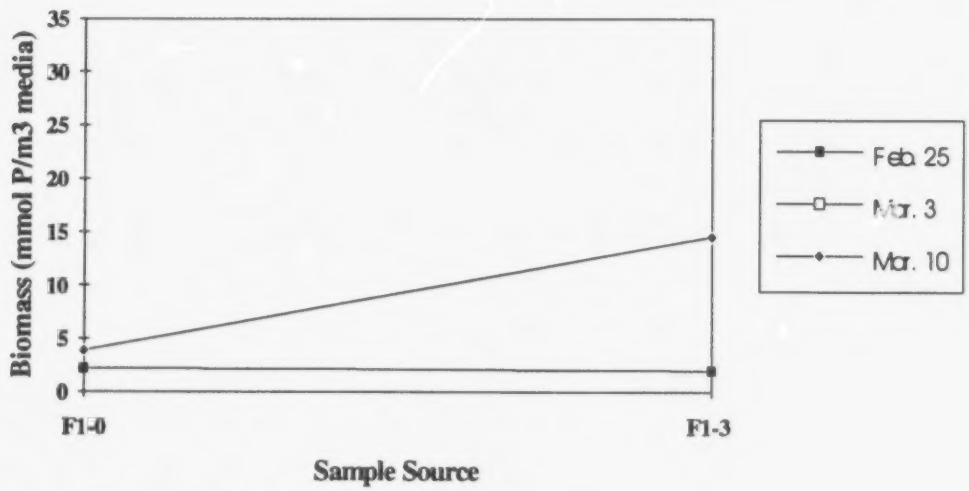


Figure 10. a. Filter 1 from February 25 to March 10, 1993.

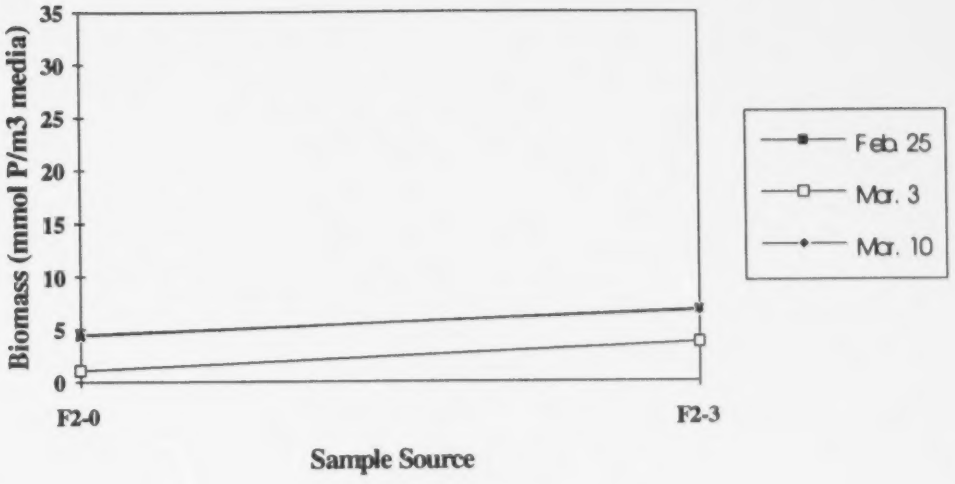


Figure 10. b. Filter 2 from February 25 to March 10, 1993.

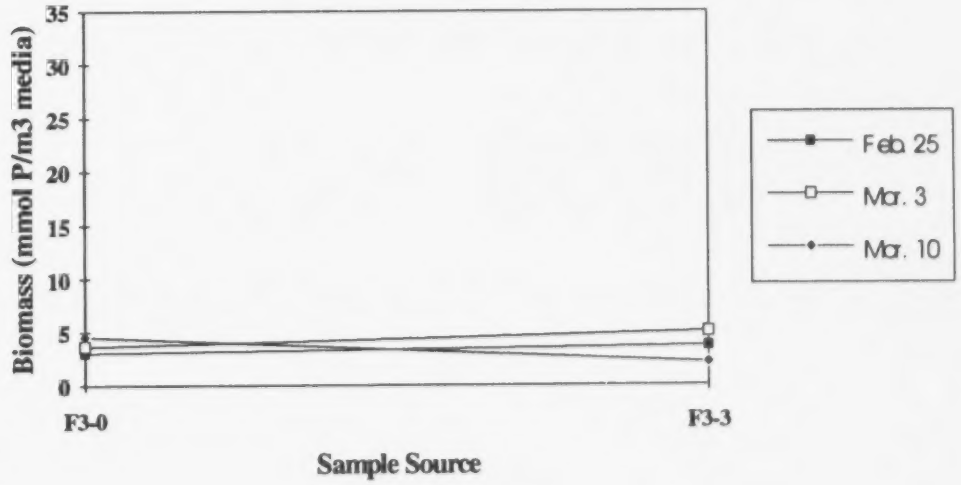


Figure 10. c. Filter 3 from February 25 to March 10, 1993.

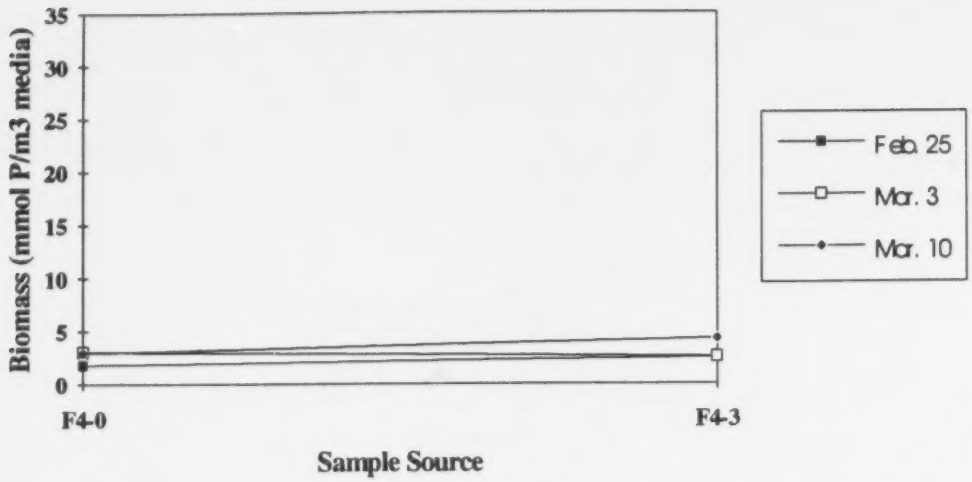


Figure 10. d. Filter 4 from February 25 to March 10, 1993.

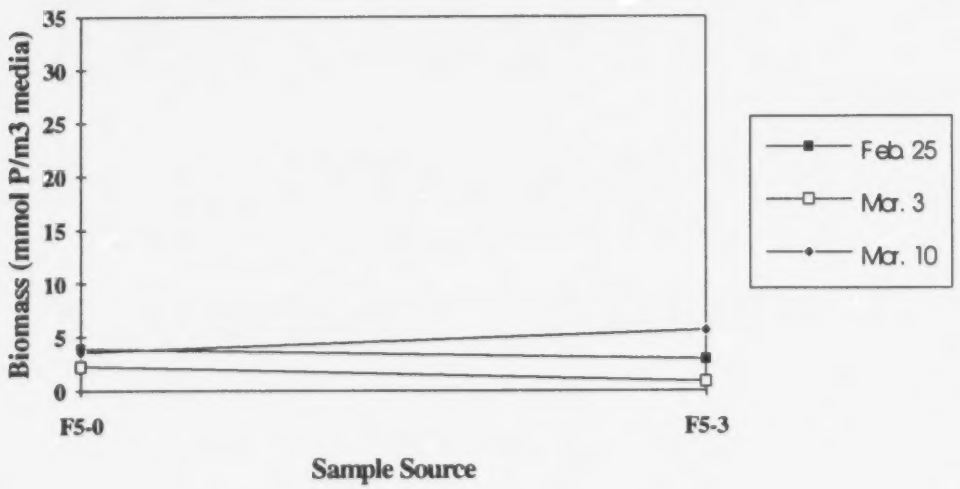


Figure 10. e. Filter 5 from February 25 to March 10, 1993.

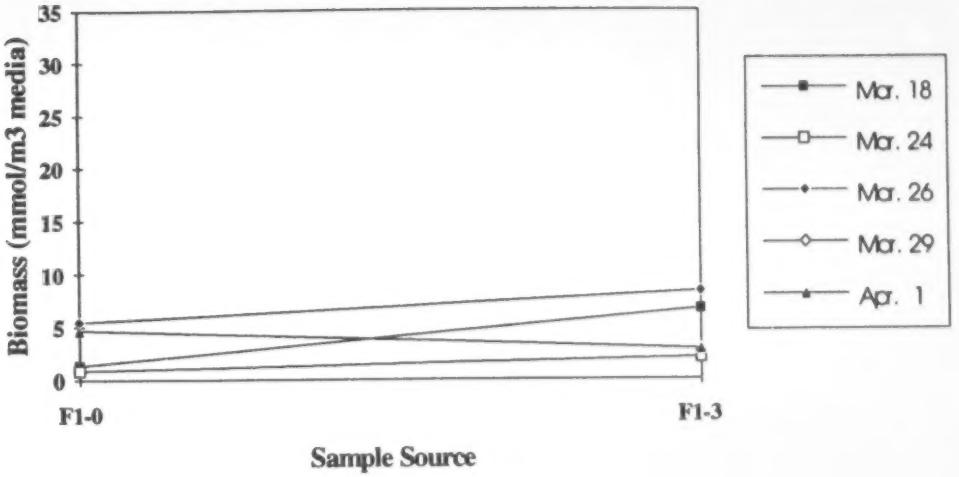


Figure 10. f. Filter 1 from March 18 to April 1, 1993.

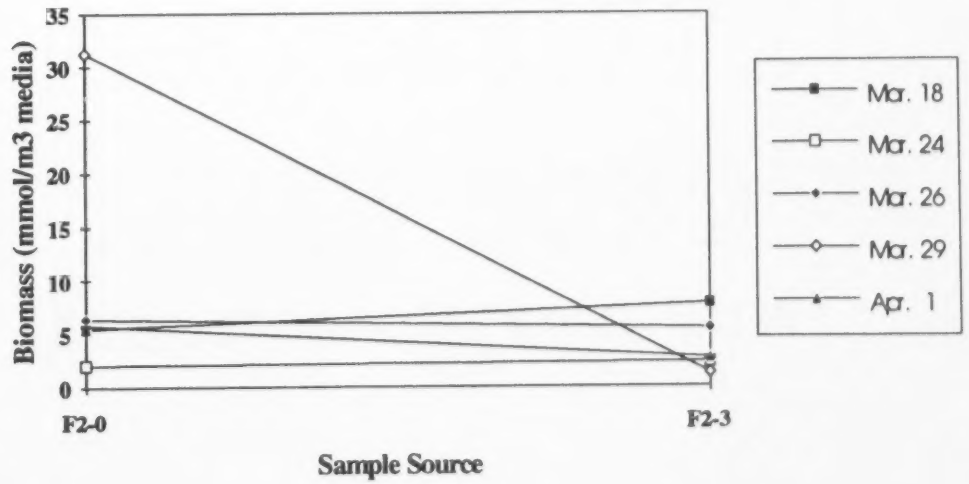


Figure 10. g. Filter 2 from March 18 to April 1, 1993.

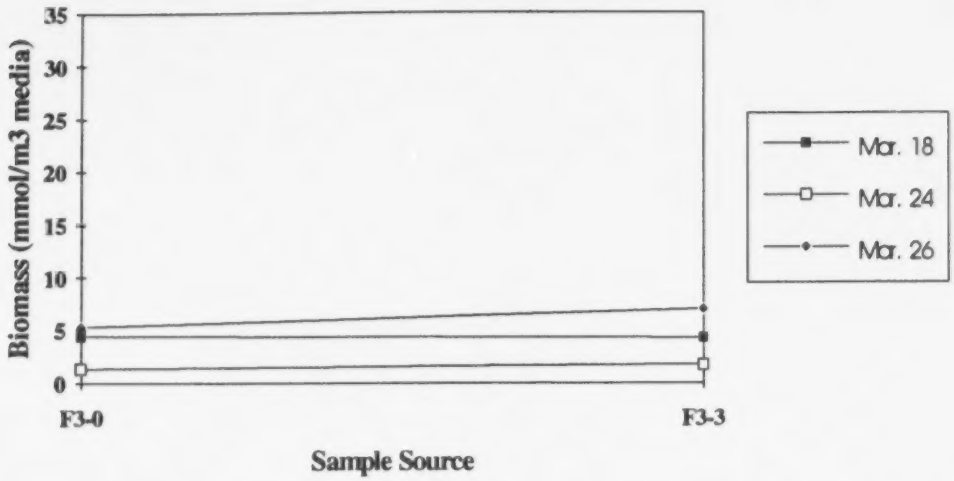


Figure 10. h. Filter 3 from March 18 to April 1, 1993.

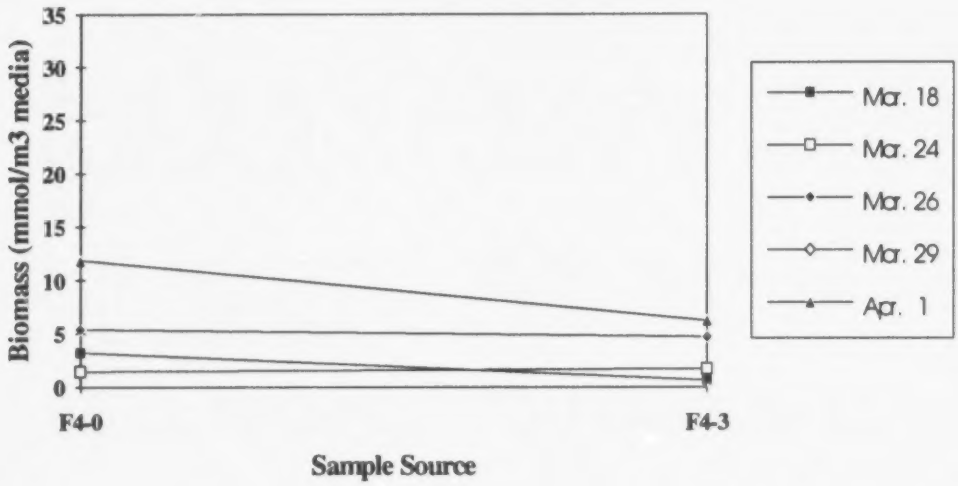


Figure 10. i. Filter 4 from March 18 to April 1, 1993.

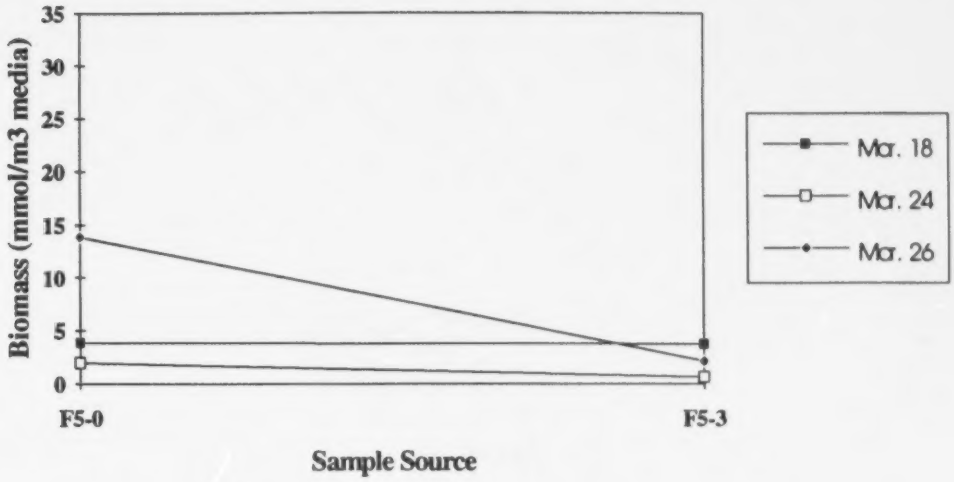


Figure 10. j. Filter 5 from March 18 to April 1, 1993.

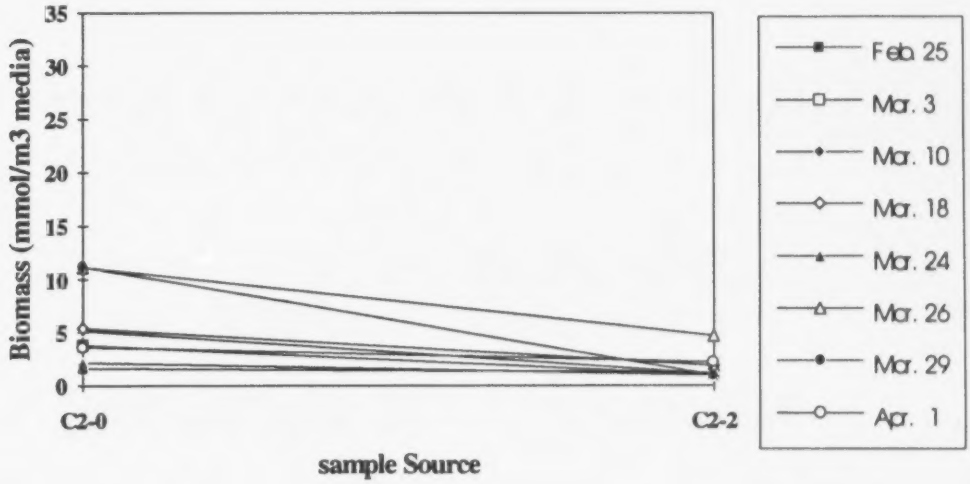


Figure 10. k. GAC2.

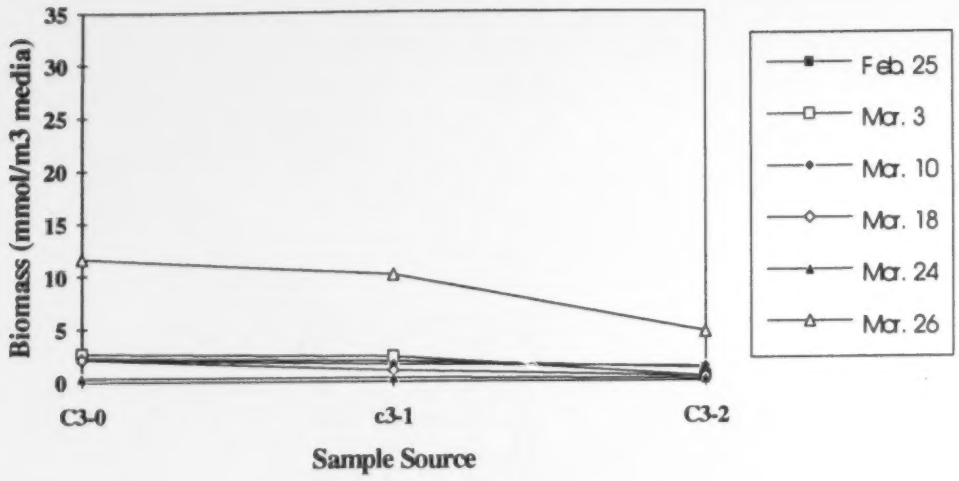


Figure 10. l. GAC3.

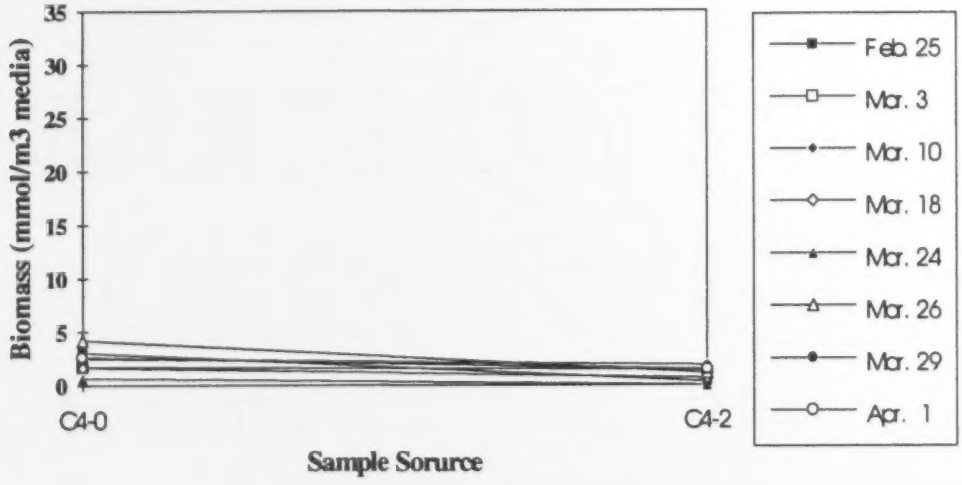


Figure 10. m. GAC4.

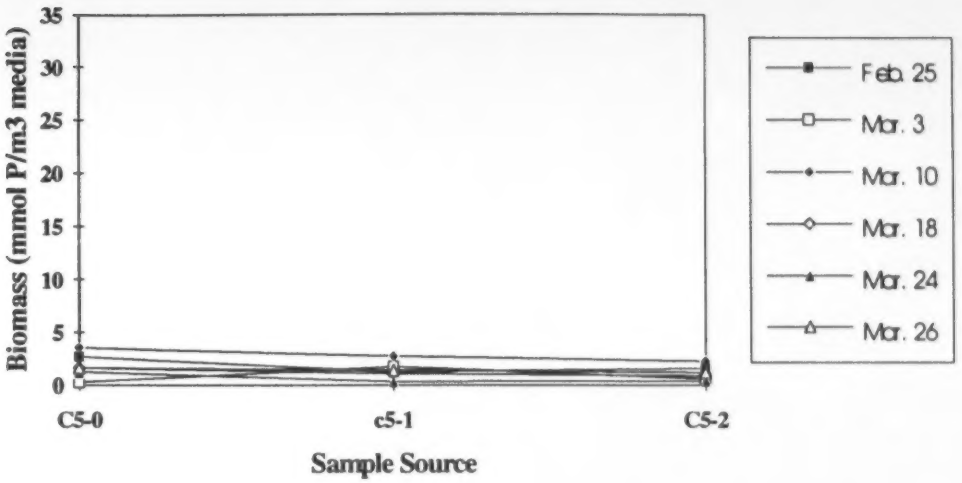


Figure 10. n. GAC5.

Figure 10 Vertical Stratification of Biomass Based on mmol P/m^3 reactor.

6.1.3 Discussion

The phenomenon that biomass decreased as a function of depth could be due to AOC removal caused by the biofilm. The data analysis showed that the biomass of the biofilm was based on the amount of AOC present in the influent, and more biofilm caused more AOC removal (see Section 6.3).

Servais et al. (1991, 1992) found that the BDOC concentration decreased along the depth of a filter, as did the biomass. A similar pattern would be expected for AOC, which would help to explain the results observed here. In this study, insufficient AOC data were available at intermediate depths to allow detailed analysis.

Prechlorination would be expected to have an effect on biofilm, too. An American Water Works Association Committee Report (1981) found that prechlorination could produce a bactericidal effect within GAC beds. The extent of the effect depends on whether free or combined chlorine is present. Dosage of chlorine above the breakpoint

produces free residual chlorine, which can be removed rapidly by passage through a GAC bed and, therefore, it may not behave as a bactericidal agent. GAC will also rapidly remove residual ozone, but anthracite will remove little if any chlorine or ozone.

In this study, Filter 1 was chlorinated to obtain a residual of 0.5 mg/L free chlorine prior to filtration. Also, Filters 2, 3, 4 and 5 received a high ozone residual from prior to February 25 until March 10. At the top of the filters (port 0), due to the bactericidal effect caused by the prechlorination or preozonation, little biofilm would be formed even though the AOC concentration was high. In the middle layer of the filters (port 2), the bactericidal effect of prechlorination or preozonation would be expected to be reduced somewhat by passage through the filter beds and the biofilm present in the top layer. (Greater removals would be expected in Filter 4, the filter-adsorber). Wende et al. (1989) showed that the biofilm environment was believed to protect cells against the activity of chlorine by diffusion resistance and neutralization of chlorine through the reaction with biofilm and pipe wall material. The AOC concentration in the middle layer could still be high because the amount of AOC removed by the small amount of biofilm present in the top layer was small. A higher amount of biofilm could be formed than in the top layer. A similar argument could be applied to port 3, near the bottom of the filter, so that, in general, little decrease of biomass with depth would be observed.

There is some decrease with depth after March 18, but the pattern for the filter-adsorber is not much different from the pattern for the other filters. The low amounts of biomass observed compared to those in another study (Cincinnati) (Miltner et al., 1993) may have precluded the development of a noticeable decrease with depth.

6.2 Effect of Operational Parameters

6.2.1 Effect of Prechlorination

Filter 1 at the pilot plant was fed with chlorinated water, and Filter 5 was fed with the water after sedimentation, without any disinfectant. Both filters received the same hydraulic loading of 10 m/h. To identify the effect of prechlorination, the biomass of Filters 1 and 5 was compared along the depth of the filter. In the sampling period from February to April 1993, samples were collected from ports 0, 2 and 3 of Filter 1 and Filter 5. At the same depth, the density of media should be essentially the same for both filters, so the results interpreted from the biomass expressed as nmol P/g media and the biomass expressed as mmol P/m^3 reactor would be the same. Therefore, only the results expressed as nmol P biomass/g media are present here. Figure 11 is the comparison of biomass between Filter 1 and Filter 5 along the depth of those two filters.

The results of duplicate analysis are shown in Figure 11, to give an indication of the experimental error in these determinations. The height of the bar represent the mean value. In general, duplicates of each sample matched very well except for a few points such as F1-0 and F5-0 on March 18, and F1-2 on March 18. Where no numbers are shown, duplicates were not available. To avoid unnecessary complication of all figures, duplicate values were only included in this figure.

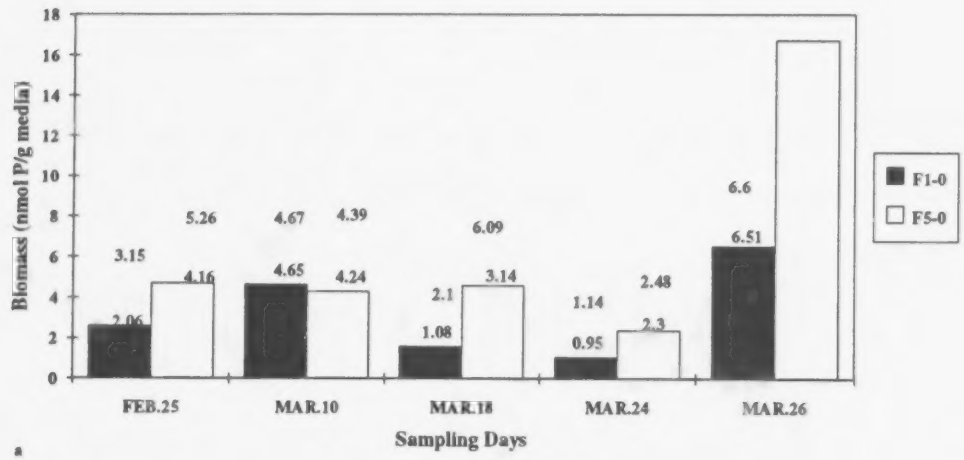
6.2.1.1 Results

From Figure 11 (a), the biomass of F1-0 (surface) was found to be lower than the biomass of F5-0 except the biomass on March 10, but a paired t-test showed that there was no significant biomass difference ($p = 0.074$) at the top of these two filters.

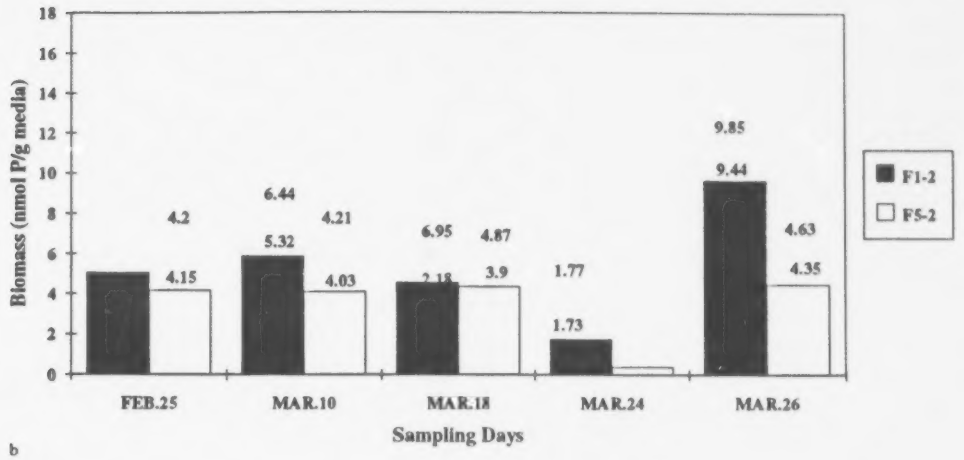
From Figure 11 (b), the biomass of F1-2 (interface) was consistently higher than the biomass of F5-2. A paired t-test showed this difference to be just significant ($p = 0.049$).

From Figure 11 (c), the biomass of F1-3 (sand layer) was higher than the biomass of F5-3 except on February 25, but this difference was just not significant ($p = 0.051$).

Therefore, prechlorination did not kill all the bacteria present in the water and did not prevent those bacteria from attaching to the media and forming biofilm. It even appeared to increase the amount of biomass.



a



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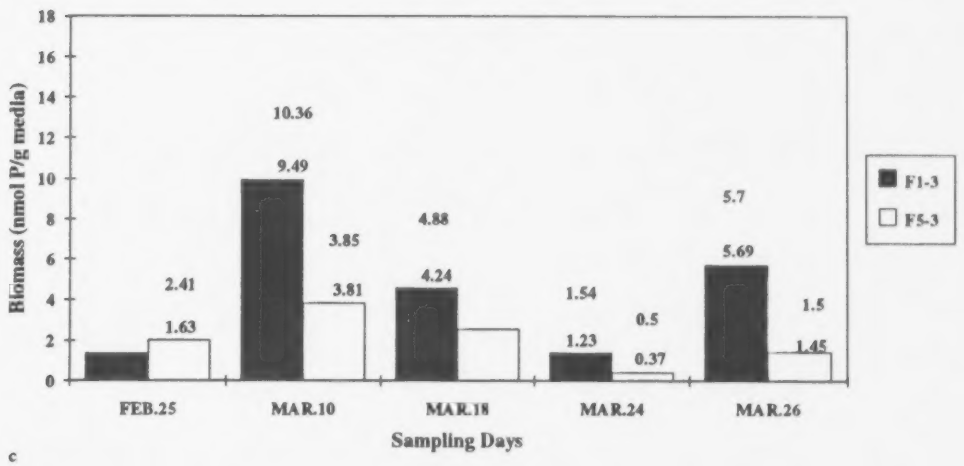


c

Figure 11 Biomass (nmol P/g media) of F1 and F5 at various depths. a. F1-0 and F5-0 b. F1-2 and F5-2, c. F1-3 and F5-3.

6.2.1.2 Discussion

Although chlorine is a very effective disinfectant and is widely used as a disinfectant in water treatment plants in North America, there are a few bacteria which are less susceptible to chlorine. Wende et al. (1989) found that biofilm and planktonic cells isolated from a RTS (Roto Torque Reactor (RT) System) consisting of four RTs in Series

at a high chlorine concentration after 38 days respesented only a few species. An estimated 75-90% belonged to one species of the genus *Pseudomonas*. Wolfe et al. (1985) found that these bacteria can be highly chlorine tolerant. The colonies of this species, on R2A-agar, were clearly the "smooth type", indicating the production of significant amounts of extracellular polymeric substances (EPS).

The HPC measurements of the present study have shown that after chlorination, there were still a few bacteria present in the influent of Filter 1 (Table 9). Those bacteria which were still alive after prechlorination could accumulate selectively on the surface of media, reproduce themselves and produce EPS to form biofilm. Experiments have shown that when the environmental conditions were bad for bacteria, they will accumulate on support media and after their accumulation, their growth can be enhanced significantly (Davies and McFeters, 1988). After bacteria have attached to the media, the chlorine may not have been efficient enough to kill them. Wende et al. (1989) stated that the biofilm environment was believed to protect cells against the activity of chlorine by increasing diffusion resistance and by neutralization of chlorine through the reaction with biofilm and pipe wall material while planktonic cells did not find such protection in their environment.

Table 9 HPC after prechlorination

Sample	HPC (CFU/mL)			
	Mar. 3, 1993	Mar. 9, 1993	Mar. 24, 1993	Mar. 26, 1993
After prechlorination	30	30	120	30

The AOC concentration following sedimentation was measured only occasionally in the later stage of the present project, because of the shortage of manpower and glassware, and because the value was quite constant in the winter season. However, the results obtained at the beginning of this project and the few data obtained in the latter

stage showed that the AOC concentration after sedimentation was much lower than the AOC concentration after prechlorination (Table 10).

Table 10 The AOC concentration of water after sedimentation and after prechlorination

Sample Source	AOC concentration (μg C/L as acetate)							
	Nov. 4, 1992	Nov. 25, 1992	Dec. 16, 1992	Jan. 13, 1993	Feb. 17, 1993	Mar. 24, 1993	Avg.	Std. Dev.
S (influent to F5)	7.1	15	12	29	9.8	5.4	13	8.5
CL (influent to F1)	19	37	20	29	24	9.5	23	9.4

At the top of the filters (port 0), the influent AOC concentration of Filter 1 was much higher than the AOC concentration in Filter 5, but it is hypothesized that the bactericidal effect caused by the prechlorination led to lower biomass than expected in Filter 1, and to no significant difference between these two filters.

In the middle layer (port 2), the bactericidal effect produced by prechlorination has been somewhat reduced by passage though the filter beds and the biofilm. The AOC concentration in Filter 1 was still probably higher than the AOC concentration in Filter 5 because the AOC removal by the low amount of biofilm at the top layer was low. A significantly higher amount of biomass could therefore be formed in Filter 1 than in Filter 5.

At the bottom layer (port 3), the bactericidal effect of prechlorination is expected to be reduced to a minimum by passage though the filter bed and the biofilm, but the AOC concentration in Filter 1 was probably low after the AOC removal caused by the biofilm present in the middle layer, and therefore little biomass was formed in the bottom layer of Filter 1. In Filter 5, the AOC concentration was low, too, because the AOC input concentration was low and part of it has been removed in the upper layers in the filter, so,

very little biofilm was formed in Filter 5. There may not be a real biomass difference between those two filters at the bottom layer.

6.2.2 Effect of Ozonation

The effect of ozonation was examined by comparing stream 2 which was fed with ozonated water, and stream 5 which was fed with the nonozonated water. They received the same hydraulic loading of 10 m/h. In the sampling period from February to April 1993, samples were collected from ports 0, 2 and 3 of Filter 2 and Filter 5, and from ports 0 and 2 of GAC 2 and GAC 5. Because biomass was compared at the same depth in each filter, the density of the media is essentially the same for both filters, so, the results interpreted from biomass expressed as nmol P/g media and as mmol P/m^3 reactor would be the same. Therefore, only the results expressed as nmol P/g media are present here. Figure 12 is the comparison between the biomass in streams 2 and streams 5 along the depth of the streams.

6.2.2.1 Results

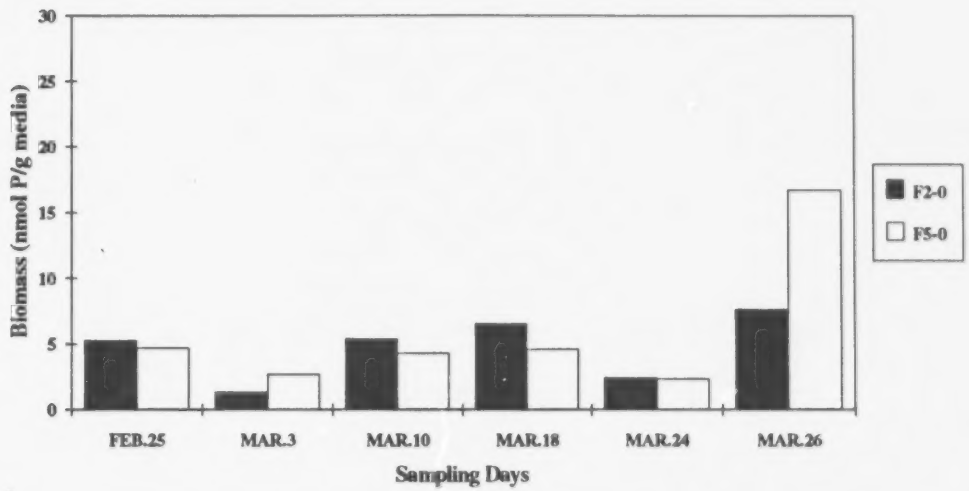
From Figure 12 (a), the biomass of F2-0 was found to be higher than the biomass of F5-0 except the biomass on March 3 and March 26, however, a paired t-test showed that there was no significant biomass difference ($p = 0.260$) between these two filters at the top layer.

From Figure 12 (b), the biomass of F2-2 was found to be consistently higher than the biomass of F5-2 except the biomass on February 25 and this difference was confirmed by a paired t-test ($p = 0.004$).

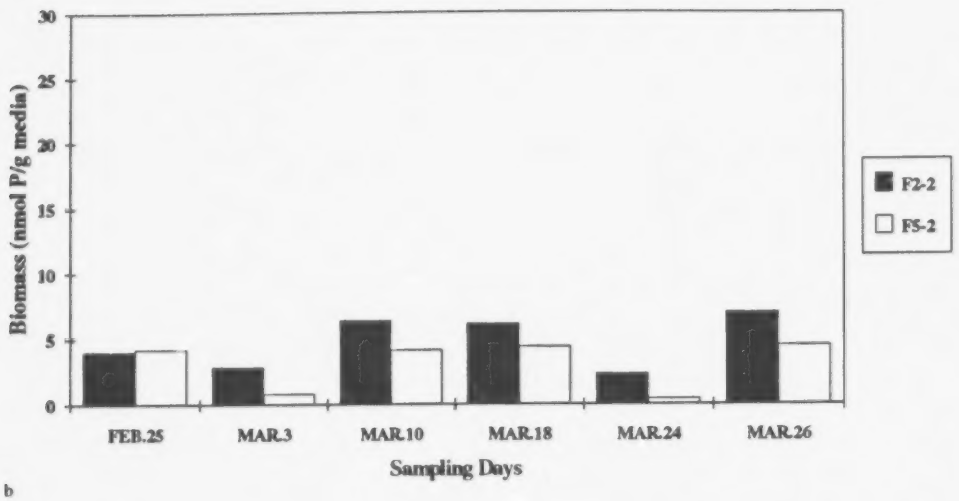
In Figure 12 (c), the biomass of F2-3 was consistently higher than the biomass of F5-3, and a paired t-test confirmed this observation ($p = 0.001$).

From Figure 12 (d), the biomass of C2-0 was higher than the biomass of C5-0 except on February 25. A paired t-test showed that the biomass in GAC contactor 2 was higher ($p = 0.048$) than the biomass in GAC contactor 5 at the top layer.

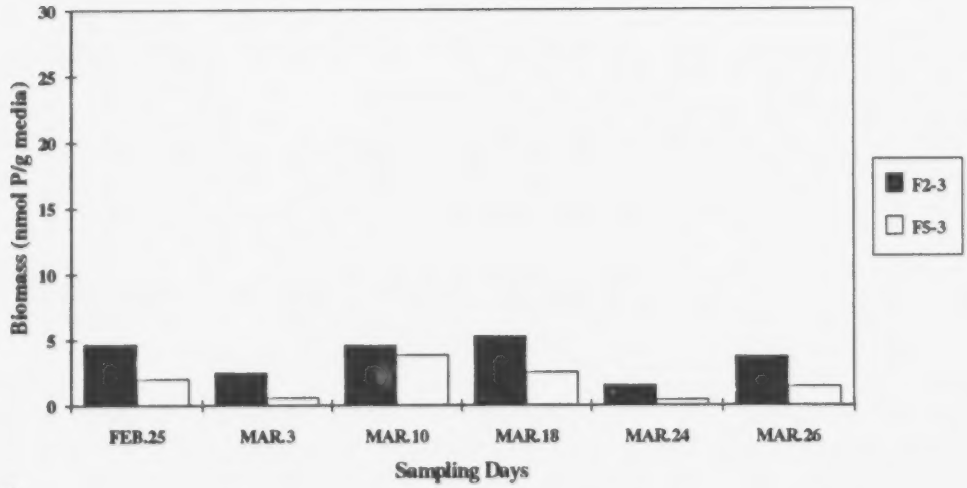
From Figure 12 (e), the biomass of C2-2 was sometimes higher than the biomass of C5-2 and sometimes lower. No significant biomass difference ($p = 0.179$) between the bottom layer of GAC 2 and GAC 5 could be found by a paired t-test.



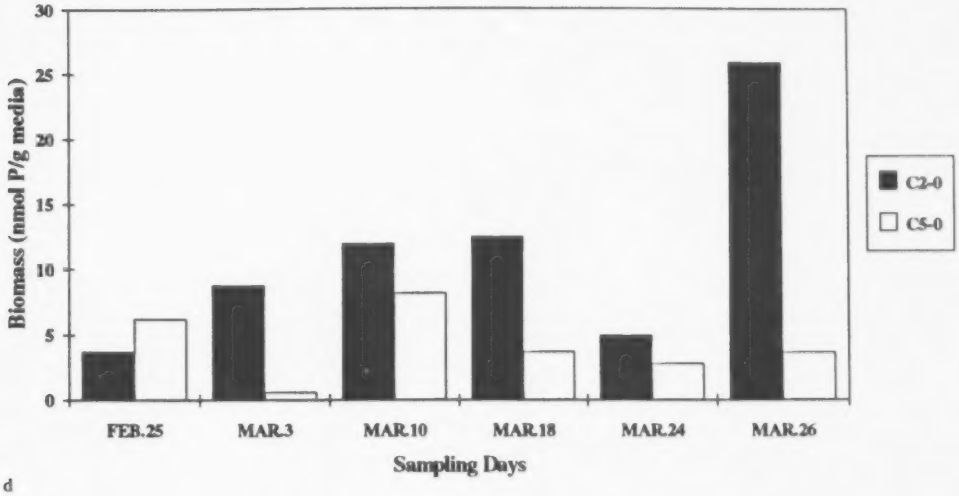
a


Biomass (nmol P/g media)
30
25
20
15
10
5
0
FEB.25
MAR.3
MAR.10
MAR.18
MAR.24
MAR.26
Sampling Days
F2-2
FS-2

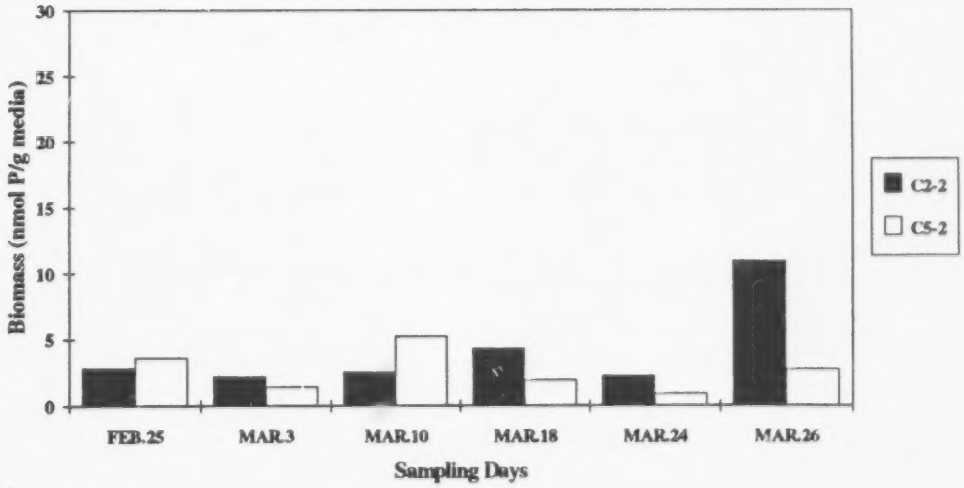
b


Biomass (nmol P/g media)
30
25
20
15
10
5
0
FEB.25
MAR.3
MAR.10
MAR.18
MAR.24
MAR.26
Sampling Days
F2-3
FS-3

c



d



e

Figure 12 The biomass (nmol P/g media) of Stream 2 and Stream 5. a. F2-0 and F5-0, b. F2-2 and F5-2, c. F2-3 and F5-3, d. C2-0 and C5-0, e. C2-2 and C5-2.

Generally, as would be expected, a higher amount of biofilm was produced in the stream which employed preozonation than in the stream which did not employ preozonation.

6.2.2.2 Discussion

The higher concentration of biodegradable organics produced by preozonation would be responsible for the greater amount of biomass present in the preozonated filter. The AOC measurements in this study showed that there was a higher AOC concentration prior to filtration in the preozonated stream than in the nonozonated stream. Due to a shortage of manpower and glassware, the AOC concentration in the nonozonated stream was only measured occasionally in the later stage of this study, but the measurements done in the early stage of the study and the few data obtained in the later stage showed that the AOC concentration after preozonation was almost always higher than the AOC concentration in the stream without preozonation (Table 11). The character of the AOC in the two streams would also be different. (It should be noted that the ozonation was turned off in early December, and therefore was not operating on December 16, 1992 and January 13, 1993. The AOC data on December 16 and January 13 were therefore omitted from Table 11.

Table 11 Influent AOC concentrations of Filter 2 and Filter 5

Sample Source	AOC concentration (μg C/L as acetate)					
	Nov. 4, 1993	Nov. 25, 1993	Feb. 17, 1993	Mar. 24, 1993	Avg.	Std. Dev.
OZ (influent to F2)	32	26	20	10	22	9.4
S (influent to F5)	7.1	15	9.8	5.4	9.3	4.2

At the top layer of filter, the AOC concentration in Filter 2 was much higher than the AOC concentration in Filter 5 (Table 11), the biomass at the top of Filter 2 should be much higher than the biomass at the top of Filter 5, but the ozone residual at the pilot plant before March 11 was very high (about 0.2 mg/L) which could cause a bactericidal effect in the filter. Therefore, even though the AOC concentration in Filter 2 was much higher than in Filter 5, the biomass at the top layer of these two filters showed no significant difference.

At the middle layer (port 2) of the filter, the bactericidal effect caused by the high residual of ozone should have been reduced somewhat by passage through the filter bed, and the AOC concentration of Filter 2 could still be much higher than in Filter 5 since the AOC removal caused by the similar range of biomass would be almost same. Therefore, a higher amount of biomass could be formed in Filter 2 than in Filter 5.

At the bottom layer (port 3), although the AOC concentration of Filter 2 has been reduced by the higher amount of biomass in the upper layers, the AOC input concentration of Filter 2 may have been sufficiently greater than that of Filter 5 that the AOC concentration present at port 3 of Filter 2 was higher than the AOC concentration at the same depth in Filter 5 (Table 12). The bactericidal effect in Filter 2 would have been reduced even more, so, a higher amount of biofilm was formed at the bottom of Filter 2 than in Filter 5.

The effluent of the two dual media filters was fed into the corresponding GAC contactor. At the top of the GAC contactor, the AOC concentration of Stream 2 was still much higher than the AOC concentration of Stream 5 (Table 12), although the AOC removal in Filter 2 was higher than in Filter 5 due to the higher amount of biomass in Filter 2 than in Filter 5. Therefore, a higher amount of biomass could be found at the top of GAC 2 than in GAC 5.

Table 12 Influent AOC concentrations of Contactor 2 and Contactor 5

Sample Source	AOC concentration (μg C/L as acetate)							
	Nov. 4, 1993	Nov. 25, 1993	Dec. 16, 1993	Jan. 13, 1993	Feb. 17, 1993	Mar. 24, 1993	Avg.	S
F2 (influent to GAC2)	33	14	3.4	8.4	36	11.9	17.8	13.4
F5 (influent to GAC5)	4.9	3.8	2.9	5.4	2.5	5.6	4.2	1.3

At the bottom of the GAC contactors, the AOC concentration of GAC 2 has been reduced to a very low value (Table 13) due to AOC removal by the higher amount of biomass in Filter 2 and the top layer of GAC 2, so, no significant biomass difference was found between GAC 2 and GAC 5.

Table 13 Effluent AOC concentrations of Filter 2 and Filter 5

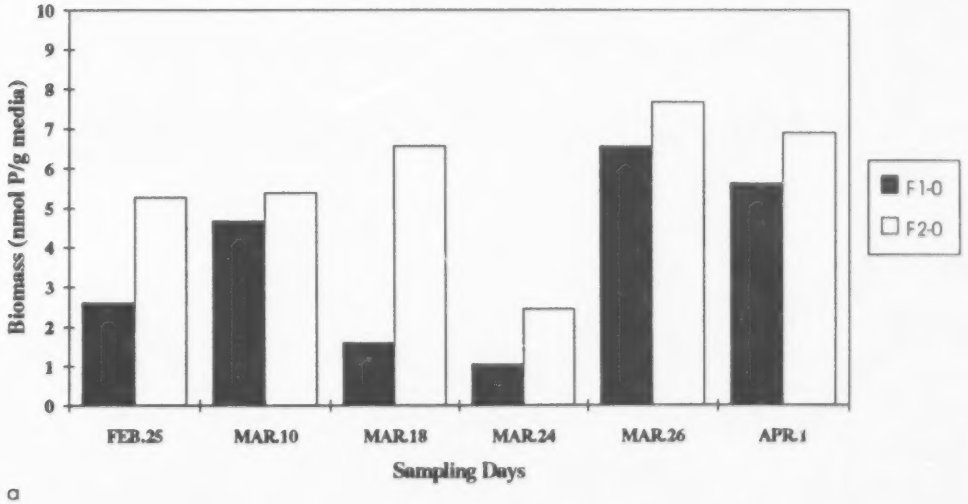
Sample Source	AOC concentration (μg C/L as acetate)							
	Nov. 4, 1993	Nov. 25, 1993	Dec. 16, 1993	Jan. 13, 1993	Feb. 17, 1993	Mar. 24, 1993	Avg.	S
F2 (influent to GAC2)	0.6	1.9	0.4	2.3	1.9	1.9	1.5	0.8
F5 (influent to GAC5)	0.6	0.7	0.4	1.9	0.2	5.7	1.6	2.1

6.2.3 Effect of Chlorination Compared To Effect of Ozonation

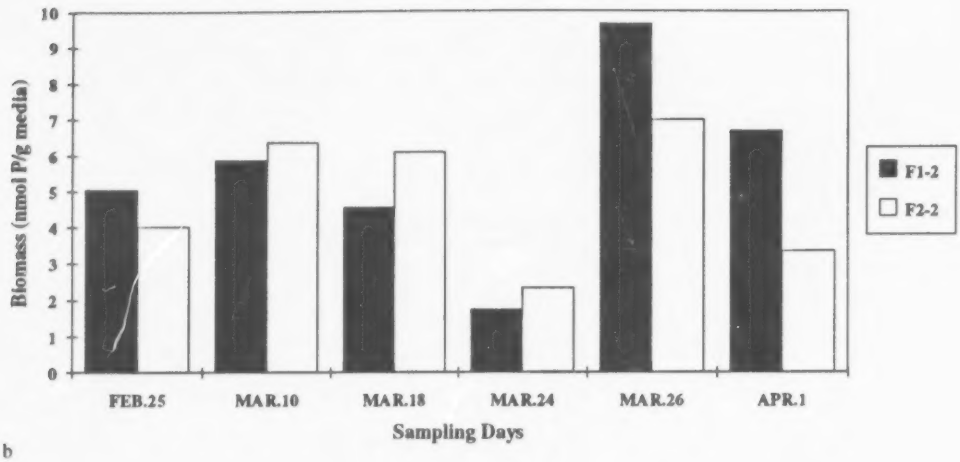
The effluent of chlorination vs ozonation was determined by comparing Filter 1 which was fed with chlorinated water and Filter 2 which was fed with ozonated water, both at the same hydraulic loading of 10 m/h. In the sample period from February to April 1993, samples were collected from ports 0, 2 and 3 of Filter 1 and Filter 2. Because biomass of Filters 1 and 2 were compared along the depth of the filter, and, at the same depth, the density of the media is essentially the same for both filters, the conclusions drawn from the biomass expressed as nmol P/g media and as mmol P/m^3 reactor would be the same. Therefore, only the results expressed as nmol P biomass/g media are present here. Figure 13 is the biomass comparison between Filter 1 and Filter 2 along the depth of those two filters.

6.2.3.1 Results

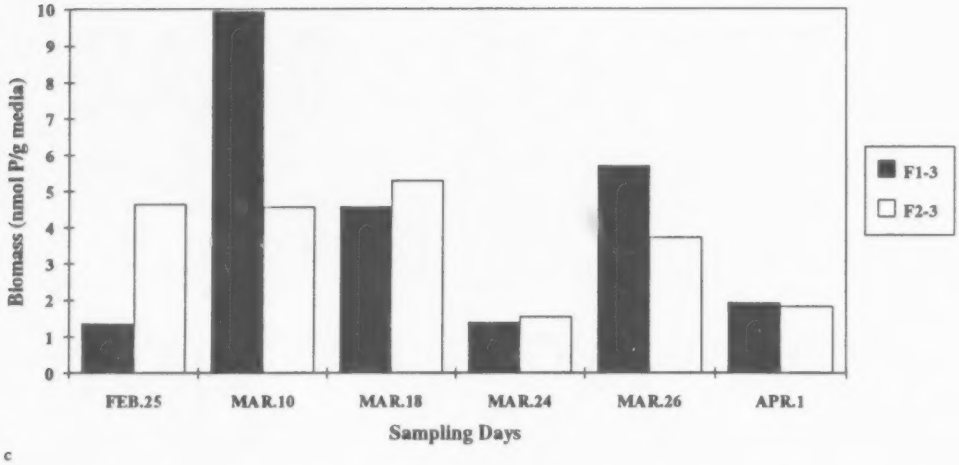
From Figure 13 (a), the biomass of F1-0 was consistently lower than the biomass of F2-0, and a paired t-test confirmed this observation ($p = 0.013$).



a



b



c

Figure 13 The biomass of F1 and F2. a. F1-0 and F2-0, b. F1-2 and F2-2, c. F1-3 and F2-3.

From Figure 13 (b), the biomass of F1-2 was sometimes higher than the biomass of F2-2, and sometimes lower. No significant biomass difference ($p = 0.201$) was found by a paired t-test, either.

From Figure 13 (c), the biomass of F1-3 was sometimes higher than the biomass of F2-3, and sometimes lower. A paired t-test showed that there was no significant biomass difference ($p = 0.333$) between these two filters.

Therefore, preozonation caused more biofilm than prechlorination, but the increased biofilm was only present at the top of the preozonated filter.

6.2.3.2 Discussion

This phenomenon that more biomass was produced in the preozonated filter than in the prechlorinated filter could be due to two reasons: 1. fewer biodegradable organics were produced by prechlorination, 2. a bactericidal effect within the filter bed was produced by prechlorination .

The AOC measurements in this study (Table 14) showed that, most of the time, the AOC concentration after prechlorination was less than the AOC concentration after preozonation. A bactericidal effect was produced by the 0.5 mg /L free chlorine residual in Filter 1. Although preozonation could produce a bactericidal effect, too, the ozone residual in the water did not produce the same effect as the chlorine residual present.

Table 14 Influent AOC concentrations of F1 and F2

Sample Source	AOC concentration (μg C/L as acetate)						
	Mar. 3, 1993	Mar. 10, 1993	Mar. 24, 1993	Mar. 26, 1993	Apr. 1, 1993	Avg.	Std. Dev.
CL (influent to F1)	14	47	9.5	56	16	29	22
OZ (influent to F2)	14	62	10	83	12	36	34

At the top layer of the filter (port 0), the AOC concentration of Filter 2 was higher than the AOC concentration in Filter 1, also, due to the bactericidal effect produced by

prechlorination in Filter 1, the biomass at the top layer of Filter 2 was higher than the biomass at the top of Filter 1.

By the middle layer, the AOC removal in Filter 2 would have been higher than the AOC removal in Filter 1 because more biomass caused more AOC removal, and the AOC concentration in Filter 2 would have been reduced. The bactericidal effect produced in Filter 1 by prechlorination would have been reduced by passage through the filter beds. These phenomena could have combined in such a way that no biomass difference was found between Filter 1 and Filter 2 at the middle port.

At the bottom layer of the filter (port 3), the AOC concentration of both filters would be expected to be even lower, and the bactericidal effect produced by prechlorination has been reduced to a minimum. Particularly in the stages of the study less biomass was formed at this depth, and no difference between the filters was found. In fact the AOC effluent measurements for these two filters in this present study were not lower than the influent values (Table 14 vs Table 15). It was suspected that the AOC measurements of the filter influents were artificially low because of inhibition.

Table 15 Effluent AOC concentrations of F1 and F2

Sample Source	AOC concentration (μg C/L as acetate)						
	Mar. 3, 1993	Mar. 10, 1993	Mar. 24, 1993	Mar. 26, 1993	Apr. 1, 1993	Avg.	Std. Dev.
F1 (effluent of F1)	11	45	19	102	12	38	38
F2 (effluent of F2)	20	49	12	100	22	39	33

6.2.4 Effect of Hydraulic Loading

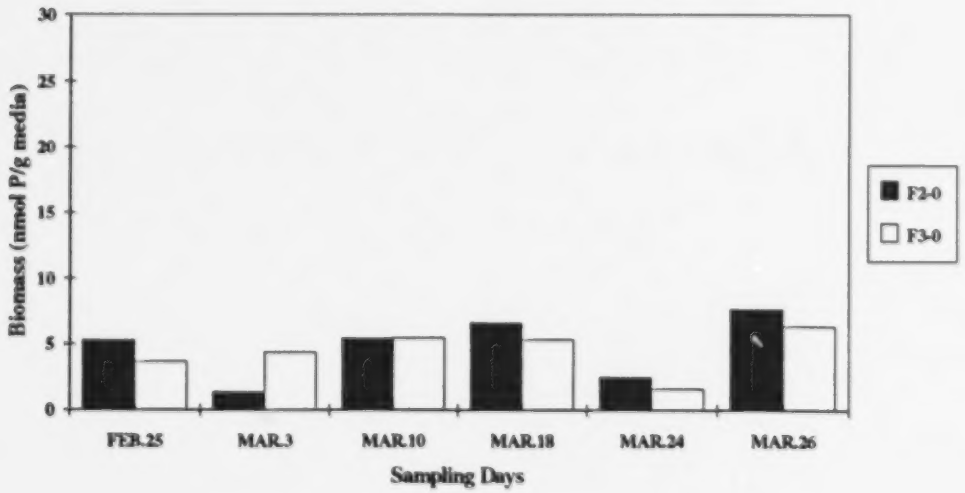
The effect of hydraulic loading was examined by comparing Streams 2 and 3 which were fed with the same ozonated water, but received different hydraulic loadings. The

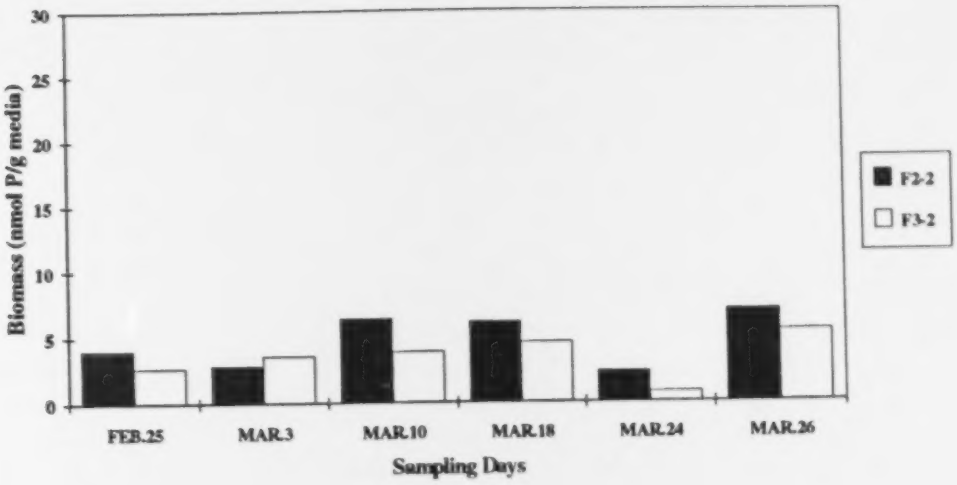
hydraulic loading of Stream 2 was 10 m/h where as that of Stream 3 was 5 m/h. Therefore, the organic flux in the bulk fluid of Steam 2 was twice the organic flux in Stream 3.

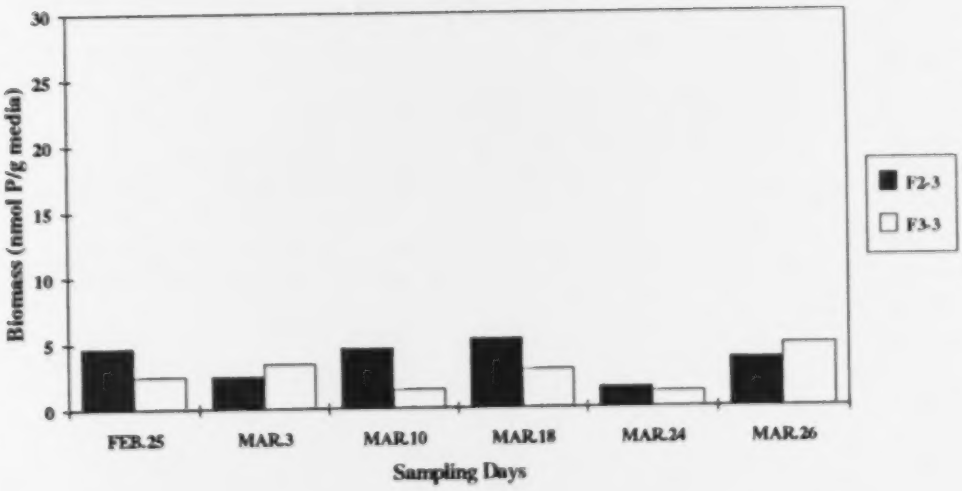
In the sampling period from February to April 1993, samples were collected from ports 0, 2 and 3 of Filter 2 and Filter 3, and ports 0 and 2 of GAC 2 and GAC 3. Because the data analysis compared the biomass of Stream 2 and Stream 3 along the depth of the stream, and because at the same depth the density of the media is almost the same for both filters, the results interpreted from the biomass expressed as nmol P/g media and mmol P/m^3 reactor would be the same. Therefore, only the results expressed as nmol P/g media are presented here. Figure 14 is the biomass comparison between Streams 2 and 3.

6.2.4.1 Results

From Figure 14 (a), the biomass of F2-0 was sometimes higher than the biomass of F3-0, and sometimes lower. No significant biomass difference between these two filters at the top layer was found by a paired t-test ($p = 0.337$).




Biomass (nmol P/g media)
30
25
20
15
10
5
0
FEB.25
MAR.3
MAR.10
MAR.18
MAR.24
MAR.26
Sampling Days
F2-2
F3-2


Biomass (nmol P/g media)
30
25
20
15
10
5
0
FEB.25
MAR.3
MAR.10
MAR.18
MAR.24
MAR.26
Sampling Days
F2-3
F3-3

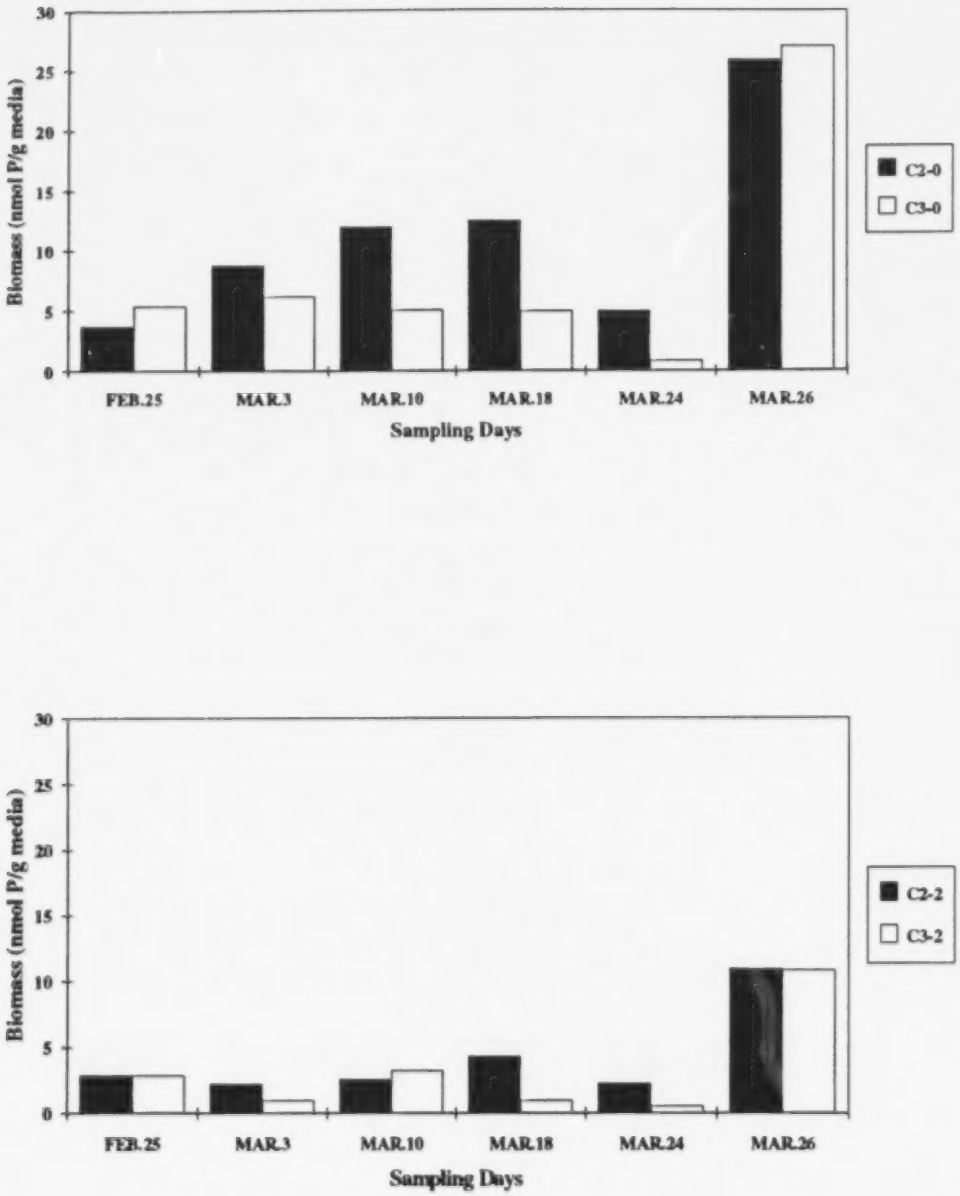


Figure 14 The biomass of Stream 2 and Stream 3. a. F2-0 and F3-0, b. F2-2 and F3-2, c. F2-3 and F3-3, d. C2-0 and C3-0, e. C2-2 and C3-2.

From Figure 14 (b), the biomass of F2-2 was found to be higher than that of F3-2 except on March 3. A paired t-test showed that the biomass in Filter 2 was significantly higher than the biomass in Filter 3 at the middle layer ($p = 0.016$).

From Figure 14 (c), the biomass of F2-3 was higher than that of F3-3 except on March 3, but a paired-t test showed no significant biomass difference between these two filters at the bottom layer ($p = 0.113$).

From Figure 14 (d), the biomass of C2-0 was found to be sometimes higher than the biomass of C3-0, and sometimes lower. No significant biomass difference between the top layers of these two contactors was found by a paired t-test ($p = 0.057$).

From Figure 14 (e), the biomass of C2-2 was sometimes higher than that of C3-2, and sometimes lower. A paired t-test showed that there was no significant difference ($p = 0.090$) between GAC 2 and GAC 3 at the bottom layer.

Generally, a higher amount of biofilm was formed in the early stages of the stream which received the higher hydraulic loading. This pattern was more consistent from March 18 to March 26, after the ozone residual had been reduced.

6.2.4.2 Discussion

The same phenomenon as seen here has been observed by van der Kooij (1978). He found that the flow rate of water through a filter bed strongly affected the colony counts of the filter materials. Experiments showed that the colony counts of GAC from the operating at a slow rate sand filters (3.5 m/h) were one to two orders of magnitude lower than those observed in the high flowrate filter.

In the present study Filter 2 and Filter 3 were fed with the same influent water, so, the AOC concentration was the same at the top of both filters, although, the flux of AOC

in Filter 2 was two times higher than in Filter 3. However, the ozone residual of both filters was very high before March 18 and produced a bactericidal effect within filter beds, which could explain why no biomass difference could be found between Filter 2 and Filter 3 at the top layer.

In the middle layer, the bactericidal effect caused by the high ozone residual would have been reduced somewhat. More biomass would be produced in Filter 2 than in Filter 3 because the AOC loading of Filter 2 was higher than the AOC loading of Filter 3.

In the bottom layer, the AOC concentration of both filters would be low. Although the concentration in Filter 2 was higher than in Filter 3 (Table 16), this difference did not lead to a significant difference in biomass. As discussed earlier, some inhibition of the AOC assay for filter influent samples is suspected, beacuse it would appear that there was a slight increase in AOC through Filter 2 (Table 16 vs Table 17).

In the GAC contactors, despite the fact that the AOC loading of GAC 2 was higher than the AOC loading of GAC 3 (Table 16), no biomass difference was found between GAC 2 and GAC 3 along the depth of the contactors.

Table 16 Effluent AOC concentrations of F2 and F3

Sample Source	AOC concentration (μg C/L as acetate)					
	Nov. 4, 1992	Nov. 25, 1992	Feb. 17, 1993	Mar. 24, 1993	Avg.	Std. Dev.
F2 (effluent of F2)	33	14	36	12	24	13
F3 (effluent of F3)	5.1	7.6	6.5	7.0	6.7	1.1

Table 17 Influent AOC concentrations of F2 and F3

Sample Source	AOC concentration (μg C/L as acetate)					
	Nov. 4, 1992	Nov. 25, 1992	Feb. 17, 1993	Mar. 24, 1993	Avg.	Std. Dev.
OZ (influent to F2 and F3)	32	27	20	10	22	9.5

6.2.5 Effect of Media

Streams 3 and 4 at the pilot plant were fed with the same ozonated water, and operated at the same hydraulic loading, but the media in stream 3 was different from stream 4. The media in Filter 3 was anthracite-sand, whereas in Filter 4 it was GAC F300-sand; in GAC 3 the carbon was F-400 whereas in GAC 4 it was Pica carbon.

In the sampling period from February to April 1993, samples were collected from ports 0, 2 and 3 of Filters 2, 3, 4 and 5, ports 0 and 2 of GAC 3 and 4.

To find the difference between anthracite and GAC F300 and between GAC F400 and Pica, the biomass value for stream 3 were compared to those in stream 4. However, because the media were the same (sand) at the bottom of Filters 3 and 4, the biomass in the bottom layer of these two filters was not be used to examine the effect of media. To identify the difference between GAC and sand, the biomass on the bottom layer of each filter was compared to the biomass at the top of each corresponding GAC contactor. It was assumed that only a small change in AOC concentration would occur between the bottom of the filter and the top of the ensuing GAC contactor.

The first results presented are the measured values expressed as nmol P/g media. However, because the density of all three media are different, the biomass expressed as mmol P/m^3 reactor was used to complete the data analysis. These values were calculated using the bulk densities presented previously. Because, the density of the Pica carbon was

not available, it was assumed to be the same as the density of F400, so the results based on mmol P/m^3 reactor for identifying the difference between GAC-F400 and Pica was the same as the result based on nmol P/g media.

6.2.5.1 Results

6.2.5.1.1. Analysis based on nmol P biomass/g media

Figures 15 a, b, c and d are the biomass of Streams 3 and 4 and Figure 15s e, f, g and h are the biomass at the bottom of each dual media filter and the biomass at the top of the corresponding GAC contactor.

From Figure 15 (a), the biomass of F3-0 was found to be consistently lower than the biomass of F4-0, and a paired t-test confirmed this observation ($p = 0.016$).

From Figure 15 (b), the biomass of F3-2 sometimes was higher than the biomass of F4-2, and sometimes lower. No significant difference between the biomass in the middle layer of Filters 3 4 was found by a paired t-test ($p = 0.203$).

From Figure 15 (c), the biomass of C3-0 was sometimes higher than the biomass of C4-0, and sometimes lower. A paired t-test showed that there was no significant difference between the biomass at the top layer of GAC contactors 3 and 4 ($p = 0.180$).

From Figure 15 (d), the biomass of C3-2 was sometimes higher than the biomass of C4-2, and sometimes lower. No significant difference between GAC 2 and GAC 4 at the bottom layer was found by a paired t-test ($p = 0.266$).

From Figure 15 (e), the biomass of F2-3 was lower than the biomass of C2-0 except on February 25. A paired t-test showed that the biomass at the bottom of Filter 2 was lower than the biomass at the top of GAC 2 ($p = 0.010$).

From Figure 15 (f), the biomass of F3-3 was found to be lower than the biomass of C3-0 except on March 24. However, a paired t-test showed that there was no significant difference ($p = 0.082$).

From Figure 15 (g), the biomass of F4-3 was always lower than the biomass of C4-0, and a paired t-test showed that the biomass at the bottom of Filter 4 was lower than the biomass at the top of GAC 4 ($p = 0.004$).

From Figure 15 (h), the biomass of F5-3 was found to be consistently lower than the biomass of C5-0, and a paired t-test confirmed this observation ($p = 0.009$).

Filter 3 and 4 were fed with the same ozonated water with the same flowrate, so, the AOC concentration, pH and temperature, etc., were same for both filters. The same AOC concentration in both filters should lead to the same amount of biofilm, but the biomass at the top of Filter 3 was less than the biomass at the top of Filter 4, which showed that GAC F300 was a better medium for bacteria to form biofilm than anthracite. However, this conclusion should be considered tentative pending the analysis based on biomass per unit volume of reactor in the next section. The biomass in GAC 3 was same as the biomass in GAC 4, which showed that Pica carbon was not a better medium for biofilm compared to GAC F400.

The AOC concentration at the bottom part of the dual media filters should be slightly higher than the AOC concentration at the top of the GAC contactors because part of the AOC concentration is removed by the biofilm in the bottom layer of the dual media filters. Even in this situation, the biomass at the bottom of Filter 2, Filter 4 and Filter 5 was less than the biomass at the top of GAC 2, GAC 4 and GAC 5 respectively. Therefore, F400 and Pica carbon were better media for bacteria to develop biofilm than sand. However this conclusion should really be based on biomass per unit volume of reactor, as discussed in the next section.

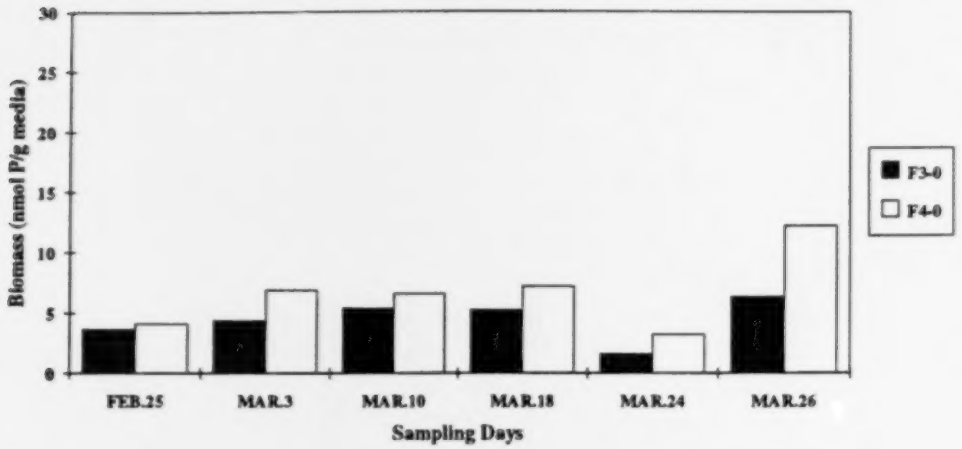


Figure 15 a. F3-0 and F4-0.

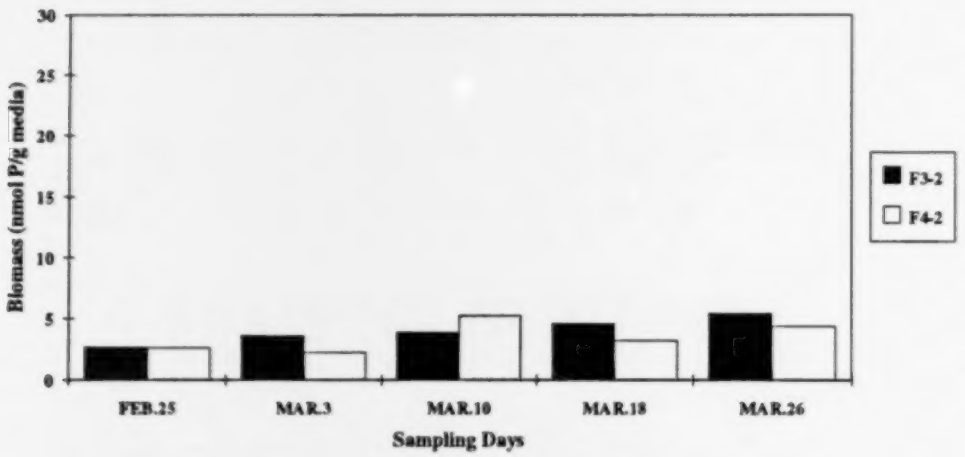


Figure 15 b. F3-2 and F4-2.

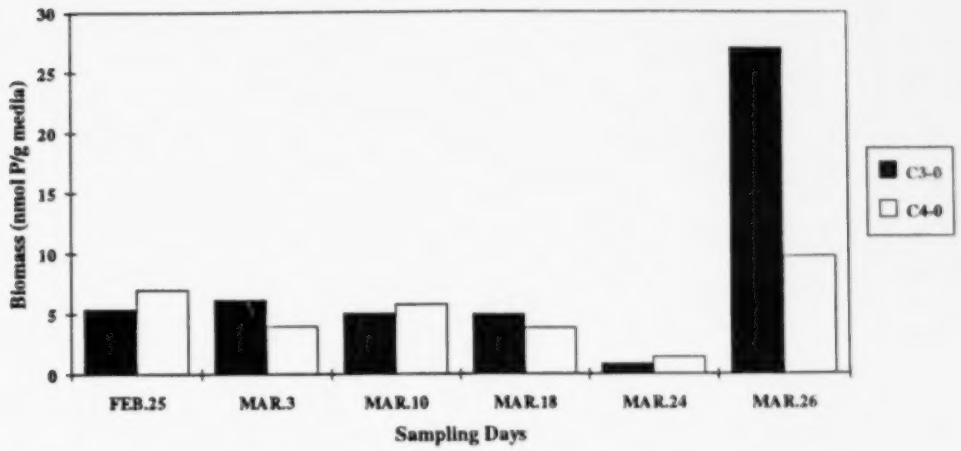


Figure 15 c. C3-0 and C4-0.

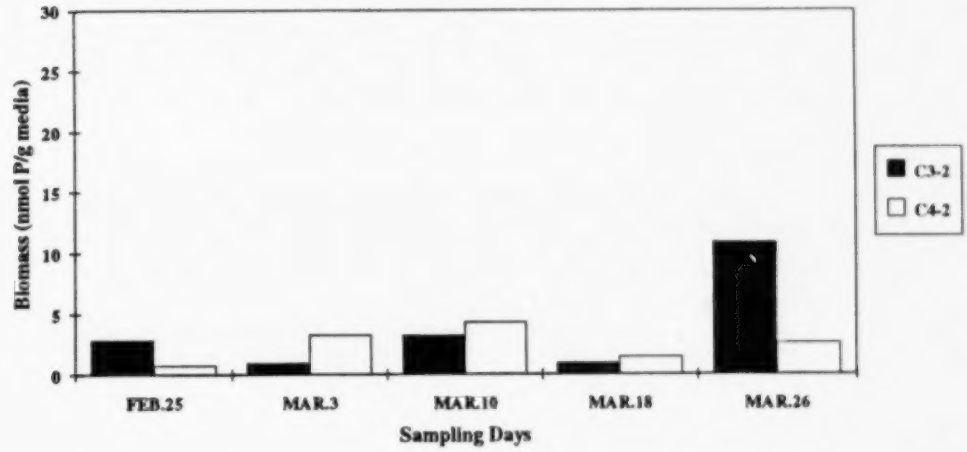


Figure 15 d. C3-2 and C4-2.

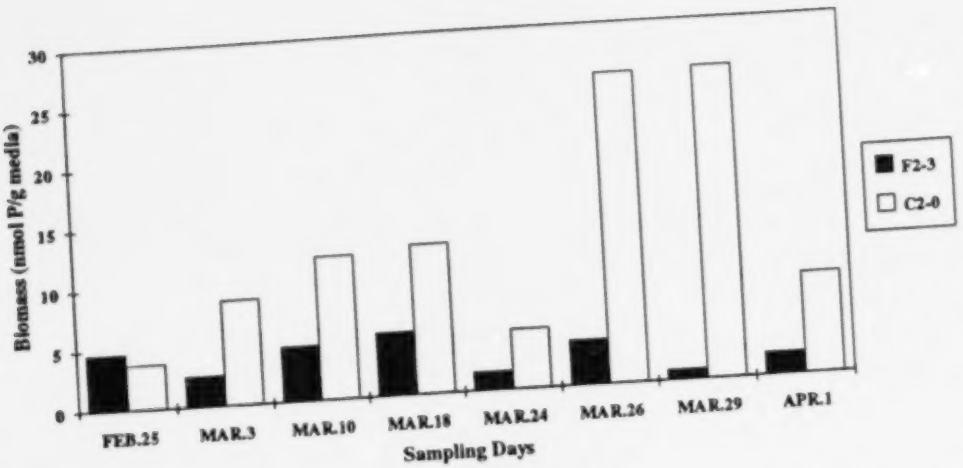


Figure 15 e. F2-3 and C2-0.

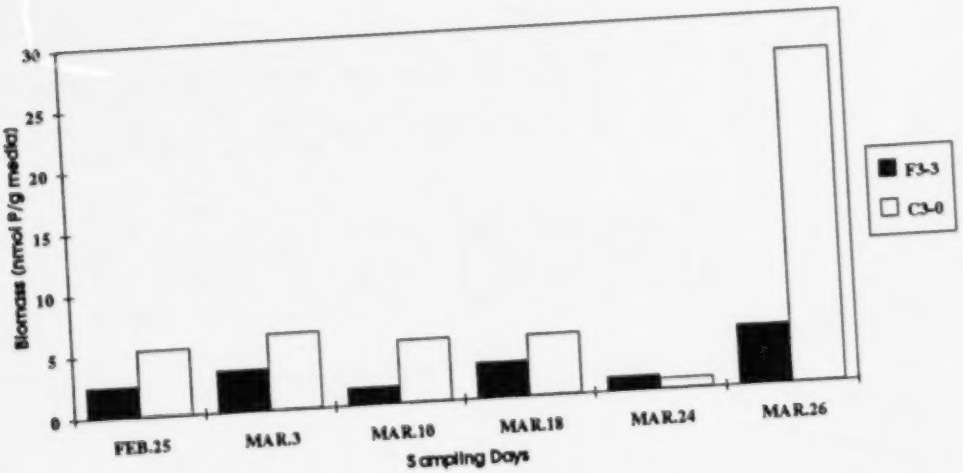


Figure 15 f. F3-3 and C3-0.

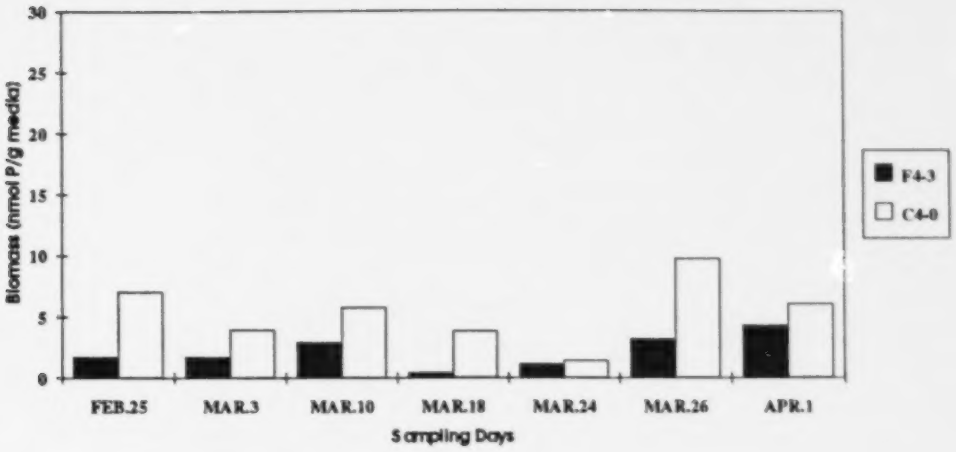


Figure 15 g. F4-3 and C4-0.

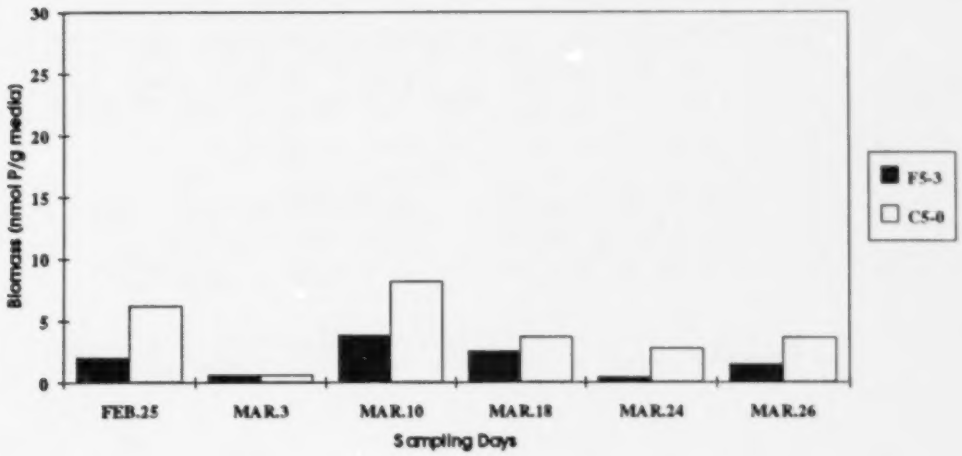


Figure 15 h. F5-3 and C5-0.

Figure 15 The biomass (nmol P/g media) of Stream 3 vs Stream 4, the biomass at the bottom of F2, F3, F4 and F5, and the biomass at the top of C2, C3, C4 and C5 respectively in units of nmol P/g media.

6.5.2.1.2. Analysis based on mmol P/m^3 reactor

Figure 16 a compares the biomass of F3-0 and F4-0, and Figure 16 b, c, d and e show the biomass at the bottom of each dual media filter and at the top of the corresponding GAC contactor.

From Figure 16 (a), it was found that, before spring runoff (March 24), the biomass of F3-0 was consistently higher than the biomass of F4-0 whereas after spring runoff the reverse was true. A paired t-test showed that the biomass of F3-0 was higher than the biomass of F4-0 ($p = 0.03$). This result is the reverse of that seen in Figure 15 (a), where the analysis was based on nmol P/g media.

From Figure 16 (b), the biomass of F2-3 was mainly higher than the biomass of C2-0 before spring runoff (March 24) whereas after spring runoff, the biomass of F2-3 was lower than the biomass of C2-0. No significant difference was found between the biomass of F2-3 and C2-0 ($p = 0.30$) using a paired t-test.

From Figure 16 (c), the biomass of F3-3 was mainly higher than the biomass of C3-0 before spring runoff (March 24) whereas after spring the reverse was true. Overall, no significant difference was found between the biomass of F2-3 and C2-0 using a paired t-test ($p = 0.36$).

From Figure 16 (d), the biomass of F4-3 was mainly higher than the biomass of C4-0, however a paired t-test showed that there was no significant difference between the biomass at the bottom of Filter 4 and at the top of GAC 4 ($p = 0.09$).

From Figure 16 (e), the biomass of F5-3 was mainly higher than the biomass of C5-0, and a paired t-test showed that there was just a significant difference between the biomass at the bottom of Filter 5 and the biomass at the top of GAC 5 ($p = 0.05$).

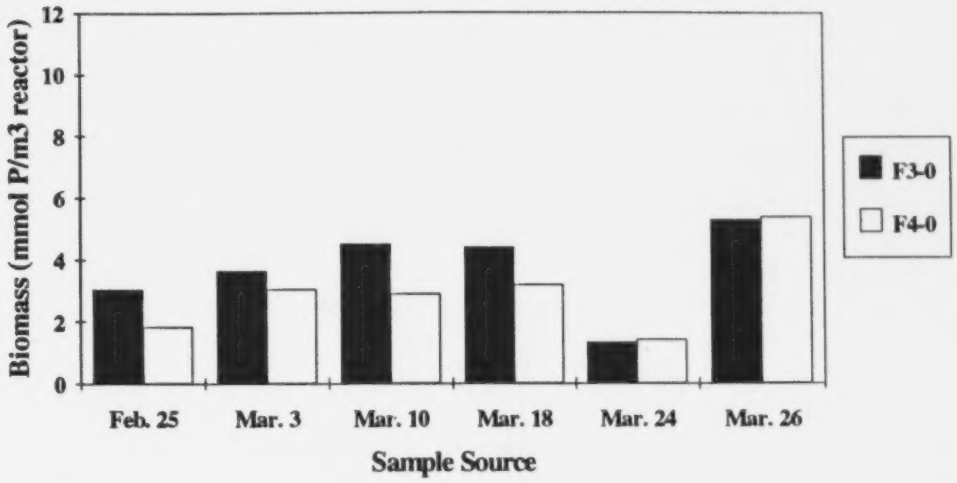


Figure 16 a. F3 and F4.

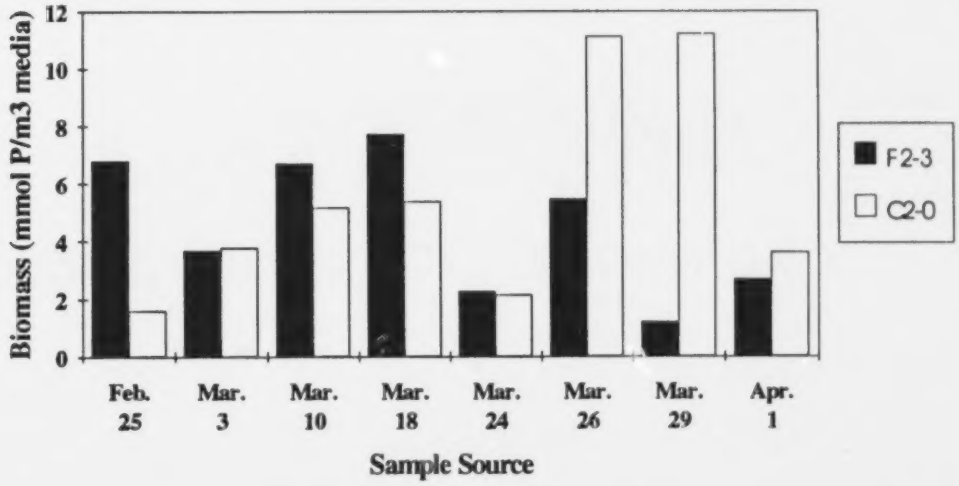


Figure 16 b. F2-3 and C2-0.

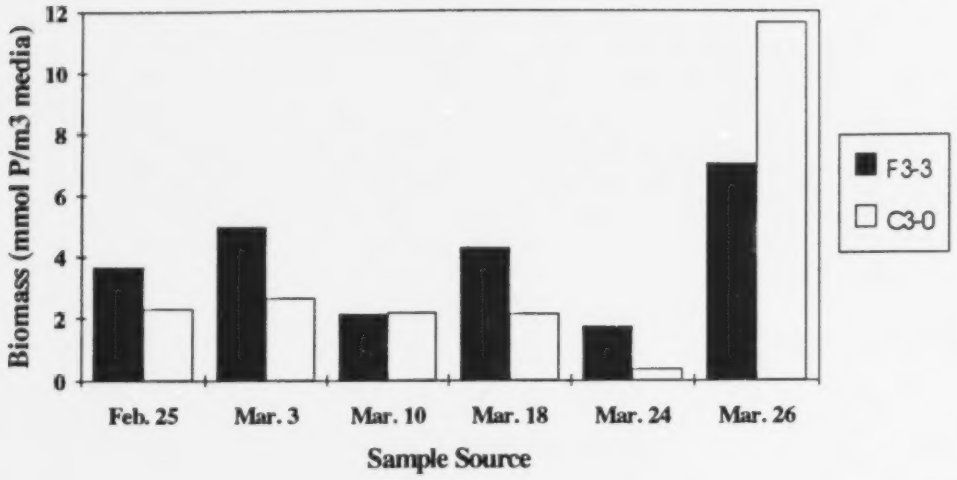


Figure 16 c. F3-3 and C3-0.

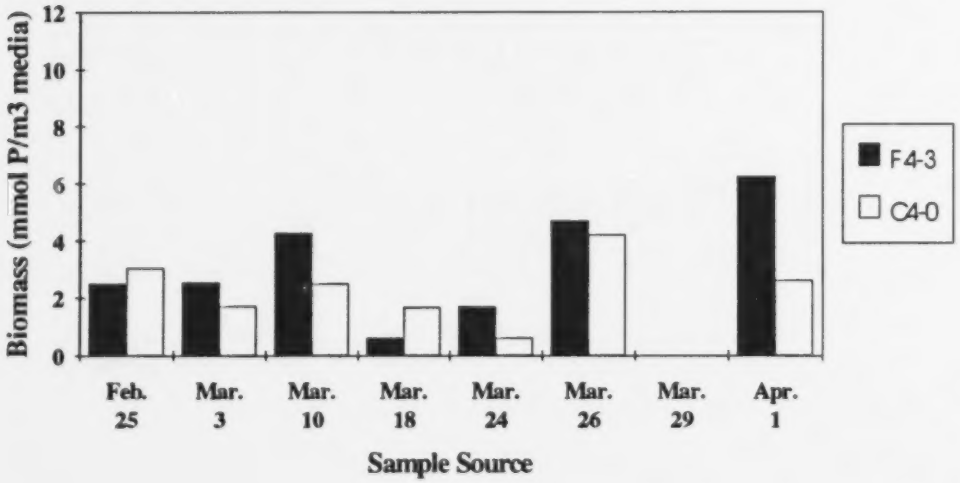


Figure 16 d. F4-3 and C4-0.

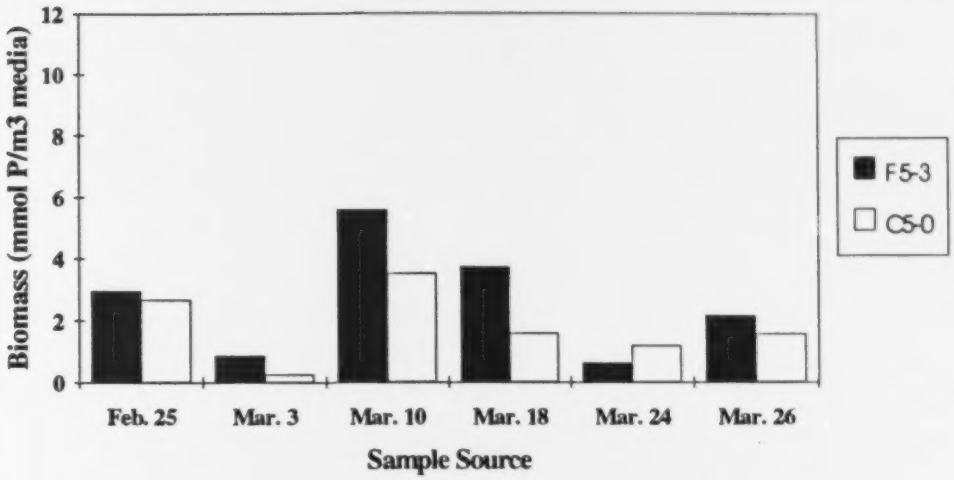


Figure 16 e. F5-3 and C5-0.

Figure 16 The biomass of Stream 3 vs Stream 4, the biomass at the bottom of F2, F3, F4 and F5 vs the biomass at the top of C2, C3, C4 and C5 in units of mmol P/m^3 reactor.

Filters 3 and 4 were fed with the same ozonated water with the same flowrate, so, the AOC concentration, pH and temperature, etc., were the same for both filters. The biomass at the top of Filter 3 (anthracite) was higher than the biomass at the top of Filter 4, which showed that the GAC F300 was not a better medium for bacteria to form biofilm than anthracite. This result is somewhat suprising.

The AOC concentration at the bottom port of the dual media filters should be slightly higher than the AOC concentration at the top of the GAC contactors because part of the AOC is removed by the biofilm in the bottom layer of the dual media filters. If AOC concentration were the controlling factor, the biomass at the bottom of Filter 2, Filter 4 and Filter 5 should have been higher than the biomass at the top of GAC 2, GAC 4 and GAC 5 respectively. This was not the case.

Although GAC-F400 and Pica carbon are not better media than sand when biomass pre unit volume of reactor is considered, during the spring runoff period, GAC-F400 did provide more biomass than sand. Therefore, the advantage of GAC may only be evident at higher AOC concentrations.

As a further refinement, biomass results could have been expressed per unit surface area, to take particle size into account. However, since none of the differences among the media were very large, this further analysis would not have contributed much.

6.2.5.2 Discussion

In their study, Ying and Weber (cited in AWWA Committee Report, 1981) proposed three possible explanations for advantages offered by GAC than sand and nonactivated carbon: (1) enrichment of oxygen by sorption, (2) enrichment of substrate in the biofilm, and (3) extended adsorption resulting from biogeneration and development of biofilms that can degrade less biodegradable but adsorbable organics.

Although as much as 19 mg/g of oxygen can be adsorbed on GAC, its availability is questionable if irreversible chemisorption occurs, a form of adsorption supported by the literature (AWWA Committee report, 1981).

With respect to the second explanation, adsorption onto GAC sites would seem to lower the substrate concentration available to the biofilm. Nevertheless, the external surface of the GAC particles will contain adsorbed and enriched organic compounds, which could promote more active biodegradation (AWWA Committee Report, 1981).

The third explanation, bioregeneration, is the most controversial. Because microorganisms have a diameter of about 1000 nm (10000 Å), they are too large to enter the micropores, which have diameters from 1 nm to 100 nm, (10 Å o 100 Å). Extracellular enzymes, however, would be able to reach substrate otherwise inaccessible.

These enzymes can biodegrade high-molecular-weight organics. Proof of their importance has been offered in studies of the attachment of film-forming marine bacteria to surfaces; 23 extracellular and intracellular enzymes were found (Corpe et al., 1972). Also, as noted by Costerton et al (1978), development of an organic slime matrix can enhance the extracellular enzyme activity of attached microorganisms. After extracellular enzyme reaction, the reaction products could diffuse to the biofilm for further degradation.

Another means of achieving bioregeneration of GAC is diffusion of sorbed substrate to the external film where biodegradation occurs. This means that desorption must occur, and, thus, the concentration gradient normally associated with adsorption must be reversed. This could happen only if microbial activity were high enough to lower the substrate concentration in the film layer and if the adsorption energy were low enough for adsorption to be a truly reversible process. Two, carefully controlled laboratory studies showed that bioregeneration occurs by this mechanism (Andrews et al., in press, Suidan et al., 1980). In both cases, however, the environment was anaerobic, the sorbed and external solution concentrations were much higher than would be found in water treatment, and the substrates (valeric acid, catechol, and phenol) were not considered strongly adsorbed. Li and DiGiano (1983) attributed higher biodegradation and specific growth rates on GAC to utilization of internally sorbed substrate. The enhanced specific growth rate increased with the concentration of sorbed substrate and with decreasing particle size, suggesting that the rate of internal diffusion was important.

There are also suggestions that bioregeneration does not occur. Van der Kooij (1978) found that the colony counts of GAC were usually larger than those of GNAC (granular-non-activated carbon) and sand. He stated that the adsorption of organic compounds by the activated carbon is not the cause of the high colony counts as usually observed in GAC-filters, and the majority of microorganisms isolated from GAC were only able to grow on simple non-adsorbing compounds like acetate, pyruvate and lactate,

whereas adsorbing substances like aromatic compounds were not utilized. The phenomenon is probably due to the relatively large surface area per unit volume of the GAC, on which microorganism utilizing substrates from the passing water can attach (The AWWA Committee Report, 1981).

Table 18 summarizes the comparisons for both ways of analyzing the data. Overall, it is not clear that GAC is superior to sand or anthracite, as would have been expected from the literature. In any of the comparisons, AOC differences were small, and therefore not a factor. The lack of a significant difference per unit reactor volume may mean that the higher surface area of the sand per unit volume (because of its smaller particle size) compensated for the superior properties of the GAC. The generally low AOC values except during spring runoff may have contributed to the lack of difference between the GAC and other media.

Table 18 Summary of Media Comparisons

	Medium Showing Higher Biomass Concentration (Significant Results, $p \leq 0.05$)	
Comparison	**nmol P/g media**	**mmol P/m^3 reactor**
Filter 3 (anthracite) vs Filter 4 (F300)		
F3-0 vs F4-0	Anthracite < F300	Anthracite > F300
F3-2 vs F4-2	NSD	NSD
C3 (F400) vs C4 (Pica)		
C3-0 vs C4-0	NSD	Not compared
C3-2 vs C4-2	NSD	Not compared
Filter vs Following GAC contactor		
F2-3 vs C2-0	Sand < F400	NSD
F3-3 vs C3-0	NSD	NSD
F4-3 vs C4-0	Sand < Pica	NSD
F5-3 vs C5-0	Sand < F400	Sand > F400

NSD: No significant difference

6.2.6 Effect of Backwashing

The dual-media filters were backwashed routinely every day in the period from February to April 1993 and air scour was employed during backwashing. The GAC contactors were backwashed every week in the same period, without air scour.

To test the effect of backwashing on bacteria fixed in the dual-media filters and GAC contactors, biomass was determined for samples collected right before and immediately after the backwashing cycle (the filters and contactors were drained immediately after backwashing to collect the samples). Samples were collected from ports 0, 2 and 3 of Filters 1, 2, 3, 4 and 5; ports 0, 1 and 2 of GAC 3 and 5; ports 0 and 2 of GAC 2 and 4. Because data analysis compared biomass of the same filter or contactor before and after backwashing, the results interpreted from the biomass expressed as nmol P/g media and as mmol P/m^3 reactor would be the same. Only the results expressed as nmol P/g media are presented here. Figure 17 is the biomass before and after backwashing in the filters and contactors.

6.2.6.1 Results

From Figure 17, the biomass before backwashing was found to be consistently higher than the biomass after backwashing for each dual media filter and GAC contactor except sample points of F1-0, F5-3 and GAC 3-2.

Statistical analysis (paired t-test) showed that the backwashing of the dual-media filters decreased the biomass significantly ($p = 0.046$), as did the backwashing of the GAC contactors ($p = 0.001$).

Figure 18 is the vertical stratification of the biomass before and after backwashing in each filter or contactor. Despite the application of air scour, the backwashing did not substantially alter the vertical distribution of the biofilm. In all cases except Filter 1, the

biomass before and after backwashing decreased as a function of depth; for Filter 1, the biomass before and after backwashing increased as a function of depth.

6.2.6.2 Discussion

Different results were observed by Servais et al. (1991). They measured the biomass just before and after backwashing of GAC filters which were washed routinely every 50-100 h of continuous operating in summer and every several days in winter. They found that there was no significant biomass loss caused by the washing and no change in the vertical stratification of the biomass. Servais et al. (1991) found that comparing bacterial enumeration of the outlet water just before and after washing the filter showed no significant difference in the exportation of bacterial biomass. These observations lead to the conclusion that washing the filter does not significantly affect microbiological function. In the present study, AOC results immediately after backwashing were not available, so this could not be assessed.

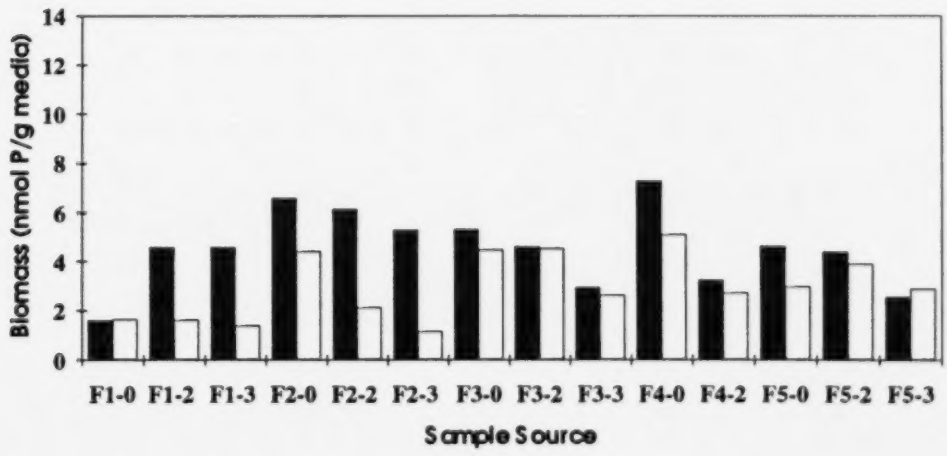


Figure 17 a. Filters

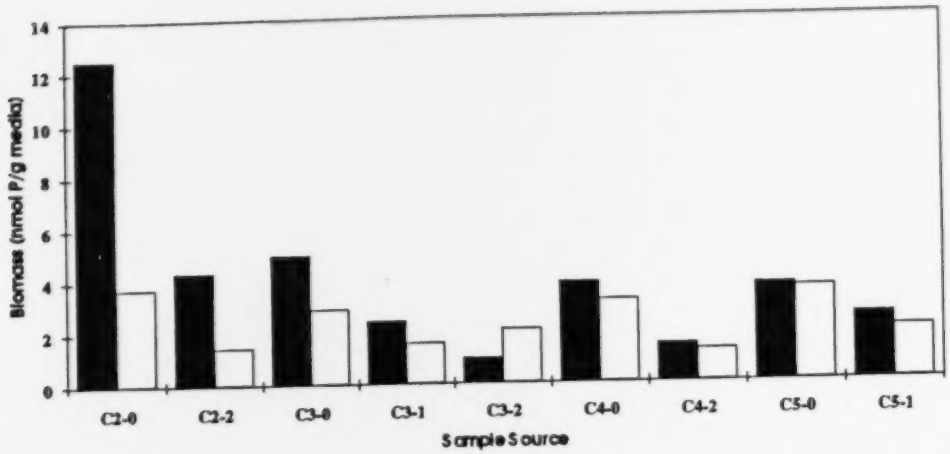


Figure 17 b. GAC Contactors

Figure 17 Biomass (nmol P/g media) Before and After Backwashing

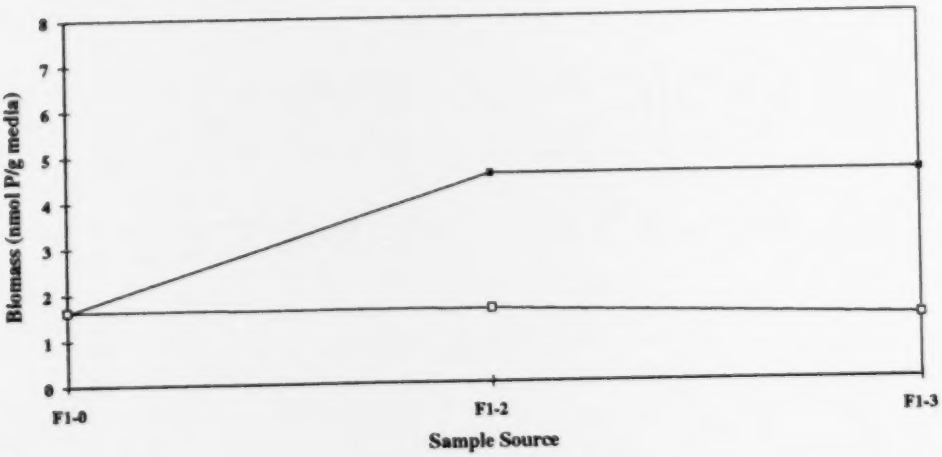
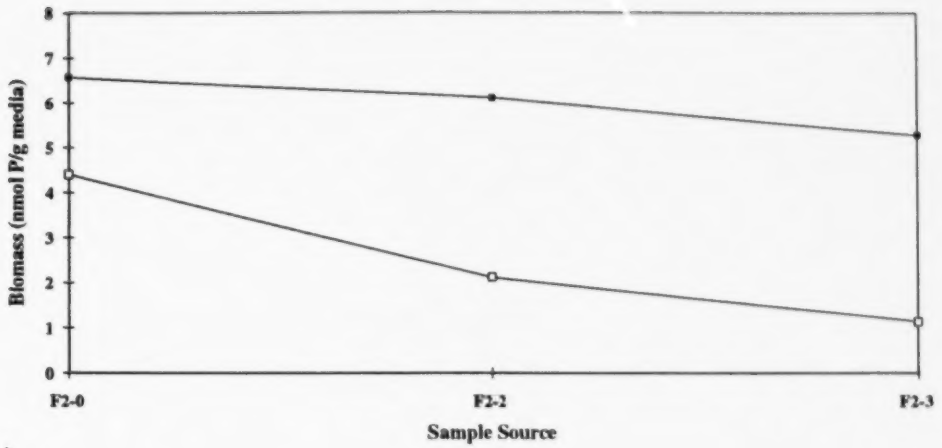
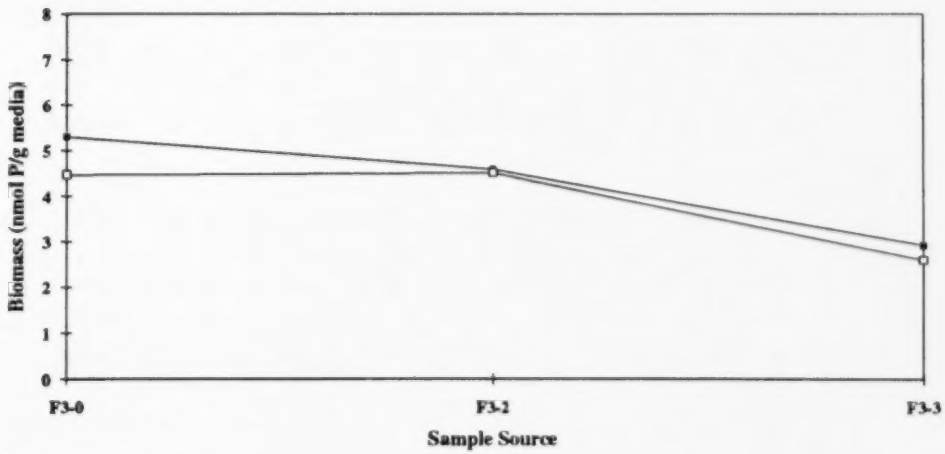


Figure 18 a. Filter 1.



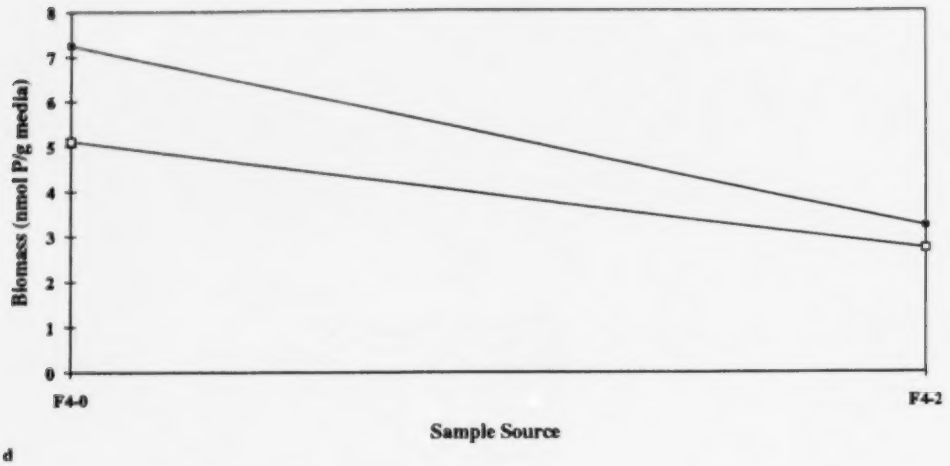
b

Figure 18 b. Filter 2.



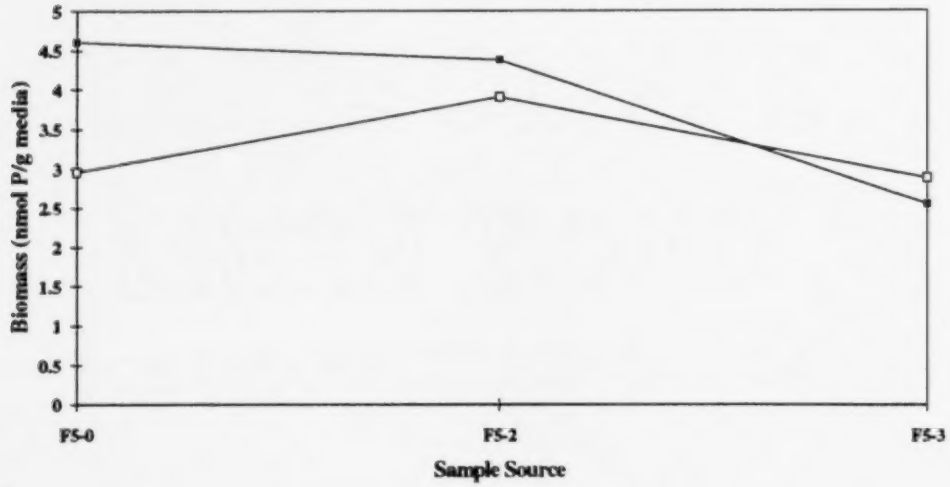
c

Figure 18 c. Filter 3.



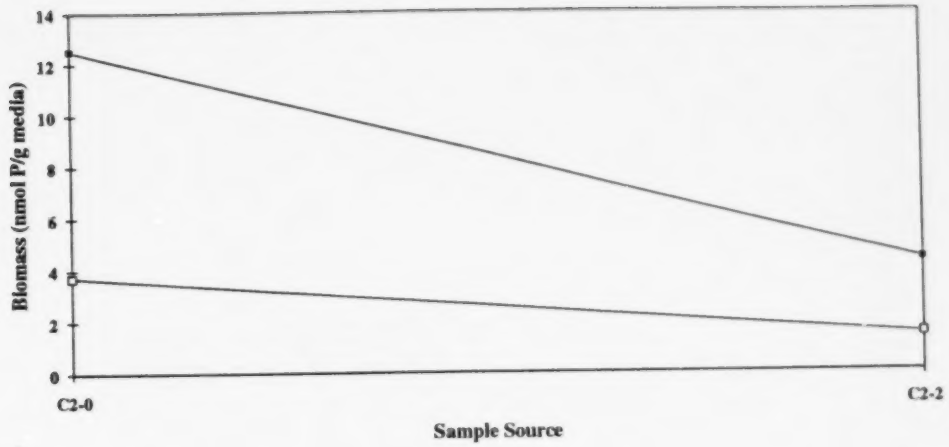
d

Figure 18 d. Filter 4.



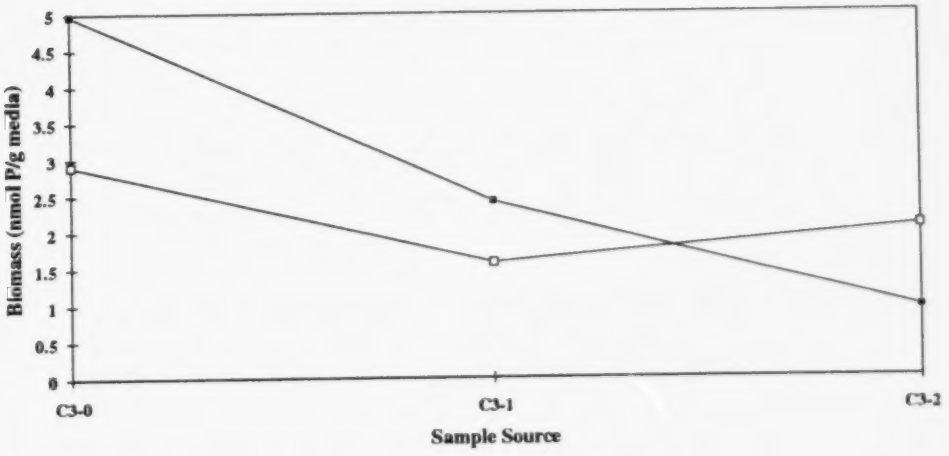
e

Figure 18 e. Filter 5.



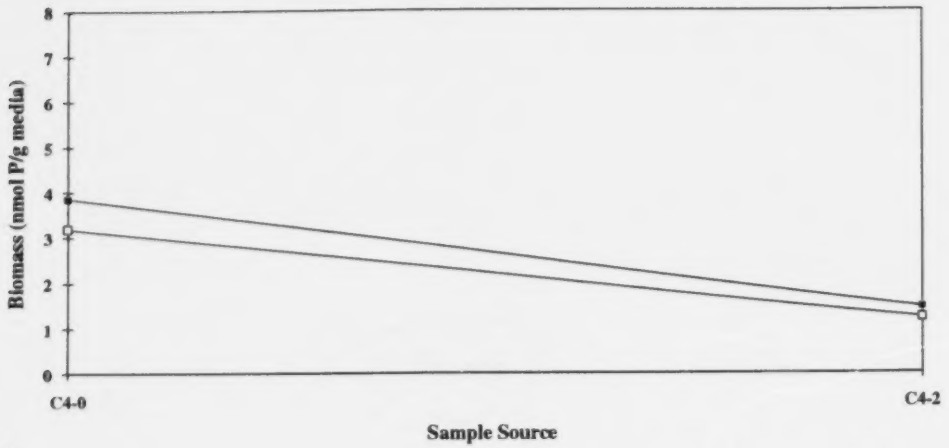
f

Figure 18 f. Contactor 2.



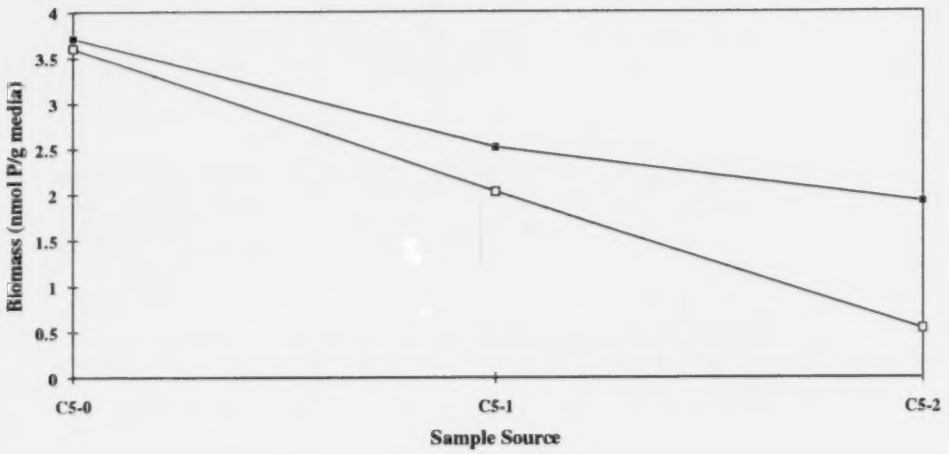
g

Figure 18 g. Contactor 3.



h

Figure 18 h. Contactor 4.



i

Figure 18 i. Contactor 5.

Figure 18 The Vertical Stratification of Biomass (nmol P/g media) Before and After Backwashing. ■: before backwashing,□: after backwashing.

6.3 Relationship Between Biomass and AOC

In the period from March to April 1993, the assimilable organic carbon (AOC) concentration in the influent and the effluent of each dual media filter and GAC contactor was measured routinely every two weeks, but during the spring runoff from March 24 to April 1, the AOC concentration was measured every three days. AOC samples were collected on March 3, 9, 24, 26 and 29 and on April 1.

The AOC removal indicated by P17 (AOC-P17) was often negative (Table 19), and therefore the AOC concentration indicated by NOX (AOC-NOX) was used to do the data analysis instead of the total AOC concentration (Table 20). Because the AOC-NOX removals in Filters 1 and 2 were often negative, only the AOC-NOX results for Filters 3, 4 and 5 and the GAC contactors were used in the data analysis.

Due to the shortage of manpower and glassware, the AOC concentrations in streams 1, 2 and 4 were measured every sampling day, but the AOC concentrations in streams 3 and 5 were measured only once.

Table 19 AOC concentration and removal indicated by P17 (AOC-P17)

Sample	AOC concentration (μg eq C/L)						AOC removal (μg eq C/L)					
Source	MAR. 3	MAR. 9	MAR. 24	MAR. 26	MAR. 29	APR. 1	MAR. 3	MAR. 9	MAR. 24	MAR. 26	MAR. 29	APR. 1
S	3.20		0									
CL1	0.20	26	0	43		0						
OZ2	0.01	14	0	8.2	3.1	0						
F1	0.90	14	0.29	52		0.02	-0.7	15	-0.3	-9.5		-0.0
F2	13	11	0.02	4	0.16	0	-13	2.8	-0	4.2	2.9	0
F3			0.06						0.1			
F4	6.5	1.2	0.2	12		1.1	-6.5	13	-0.2	-3.8	3.1	-1.1
F5			4.7						-4.7			
C2	0.8	1.7	0.25	5.4	55	1.7	12	9.2	-0.20	-1.4	-54	-1.7
C3			0.01						0.05			
C4	24	7.5	93	14		155	-18	-6.3	-93	-1.6		-154
C5			0.64						4.1			

For the removals, a blank in the table means that no sample was obtained except for the CL and OZ rows in which no value is shown.

Table 20 AOC concentration and removal indicated by NOX

Sample	AOC concentration (μg eq C/L)						AOC removal (μg eq C/L)					
Source	MAR. 3	MAR. 9	MAR. 24	MAR. 26	MAR. 29	APR. 1	MAR. 3	MAR. 9	MAR. 24	MAR. 26	MAR. 29	APR. 1
S			5.4									
CL1	14	47	9.5	56		16						
OZ2	14	62	10	83	22	12						
F1	11	45	19	102		22	2.3	2.7	-9	-46		-6.2
F2	20	49	12	92	24	24	-6	13	-1.9	-9.1	-1.6	-12
F3			7.0						3			
F4	9.6	14	5.9	52		16	4.8	47	4.1	31		-4
F5			5.6						-0.2			
C2	2.3	4.8	1.7	6.9	1.3	4.6	18	44	10	85	22	19
C3			0						7.0			
C4	4.5	9.0	2.5	8.3		5.6	5.1	5.1	2.4	44		11
C5			7.7						-2.1			

For the removals, a blank in the table means that no sample was obtained except for the CL and OZ rows in which no value is shown.

6.3.1 Results

6.3.1.1 Effect of AOC Influent Concentration

The biomass at the top of the dual media filters and GAC contactors is the biomass which responds directly to the AOC concentration in the influent. Because of the effect of high ozone residual on the filters before March 18, the analysis for the filters was conducted by dividing the data into two groups: the first one was the data collected from

March 3 to March 10, and the second one was the data collected from March 24 to April 1.

6.3.1.1.1. Correlation between biomass (nmol P/g media) and AOC influent concentration

Table 21 is the AOC influent concentration of each filter and the biomass at the top layer of each filter from March 3 to March 10, 1993; Table 22 is the AOC influent concentration of each filter and the biomass at the top layer of each filter from March 24 to April 1, 1993. Table 23 is the AOC influent concentration of and the biomass at the top layer of each GAC contactor.

From Table 23, the biomass at the top of the GAC contactors was found to be linearly related to the AOC influent concentration, and a fairly good correlation (Figure 19) was obtained ($r=0.73$), which confirmed the observation. This correlation was significant at 5% level ($\nu=11$). It should be noted, however, that the correlation is substantially helped by the one data point in the upper right corner of the figure. Such a relationship was not obtained for the filters (because the correlation in the filters was not significant, those figures were not shown).

Table 21 AOC influent concentration and biomass (nmol P/g media) at the top of each filter from March 3 to March 10, 1993.

Time	Sample					
	F3		F4		F5	
	AOC#	Biomass*	AOC	Biomass	AOC	Biomass
Mar. 3	14	4.37	14	6.92		2.73
Mar. 9	62	5.44	62	6.62		4.43

#AOC concentration, µg eq C/L, *Biomass, nmol P/g media.

Table 22 AOC influent concentration and biomass (nmol P/g media) at the top of each filter from March 24 to April 1, 1993.

Time	Sample					
	F3		F4		F5	
	AOC#	Biomass*	AOC	Biomass	AOC	Biomass
Mar. 24	10	1.59	10	3.21	5.4	2.39
Mar. 26	83	6.36	83	12.22		16.73
Mar. 29						
Apr. 1			12.06	26.95		

Table 23 AOC influent concentration and biomass (nmol P/g media) at the top layer of each GAC contactor.

Time	Sample							
	C2		C3		C4		C5	
	AOC#	Biomass*	AOC#	Biomass*	AOC#	Biomass*	AOC#	Biomass*
Mar. 3	20	8.76		6.18	9.6	3.95		0.59
Mar. 9	49	11.97		5.07	14	5.79		8.2
Mar. 24	12	4.98	7.0	0.84	5.9	1.42	5.6	2.78
Mar. 26	92	25.84		27.05	52	9.76		3.65
Mar. 29	24	26.05						
Apr. 1	24	8.36			16	6.08		

*Biomass, nmol P/g media; #AOC concentration, µg eq C/L.

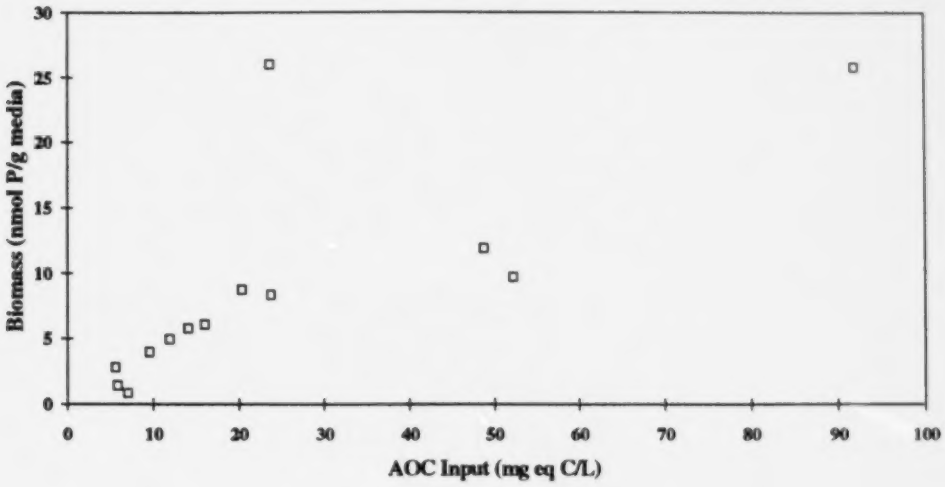


Figure 19 Correlation between the biomass in the top layer (nmol P/g media) and the AOC influent concentration for the GAC contactors

6.3.1.1.2. Correlation Between Biomass (mmol P/m^3 reactor) and AOC Influent Concentration

For the filters, the same data analysis was conducted by using mmol P/m^3 reactor instead of nmol P/g media. The analysis was not done for the GAC contactors since the pattern of the results would have remained unchanged. Table 24 is the AOC influent concentration and the biomass in the top of each filter from March 3 to March 10, 1993; Table 25 is the AOC influent concentration and the biomass in the top layer of each filter from March 24 to April 1.

There was little change in the relationship for the filters and the correlation was not improved, therefore, figures were not shown.

Table 24 AOC influent concentration and biomass in the top layer (mmol P/m^3 reactor) of each filter from March 3 to March 10, 1993.

Time	Sample					
	F3		F4		F5	
	AOC#	Biomass*	AOC#	Biomass*	AOC#	Biomass*
Mar. 3	14	3.63	14	3.05		2.27
Mar. 9	62	4.52	62	2.91		3.59

*Biomass, mmol P/m3 reactor; #AOC concentration, µg eq C/L.

Table 25 AOC influent concentration and biomass in the top layer (mmol P/m^3 reactor) of each filter from March 24 to April 1, 1993.

Time	Sample					
	F3		F4		F5	
	AOC#	Biomass*	AOC#	Biomass*	AOC#	Biomass*
Mar. 24	10	1.32	10	1.41	5.4	1.98
Mar. 26	83	5.28	83	5.38		13.89
Mar. 29						
Apr. 1			12	11.86		

6.3.1.2 Effect of Biomass on AOC Removal

The AOC removal was the difference between the concentration in the influent and the concentration in the effluent. To examine the effect of biomass on AOC removal, the average biomass of each dual media filter or GAC contactor, which was the average biomass of all the samples collected from that filter or contactor on a given day was used because this value best represented the biomass in the filter or GAC contactor. Because of the effect of the high ozone residual on the filters before March 18, the analysis for filters was conducted by dividing the data into two groups: the first one was the data collected from March 3 to March 10; the second one was the data collected from March 24 to April 1.

6.3.1.2.1. Correlation Between AOC Removal and Biomass (nmol P/g media)

Table 26 is the average biomass and the AOC removal of each filter from March 3 to March 10, 1993; Table 27 is the average biomass and the AOC removal of each filter from March 24 to April 1. Table 28 is the average biomass and the AOC removal of each contactor.

From Table 28, the AOC removal in the GAC contactors was found to be linearly related to the biomass, and a fairly good line (Figure 20) was obtained ($r=0.73$, $r_{table}=0.553$ at $\alpha=5\%$, $v=11$), which confirmed this observation. Such relationship was however not obtained for the filters, therefore, figures are not shown

Table 26 Biomass (nmol P/g media) and AOC removal of the filters from March 3 to March 10, 1993

Time	Sample					
	F3		F4		F5	
	Biomass*	ΔAOC#	Biomass*	ΔAOC#	Biomass*	ΔAOC#
Mar. 3			3.63	4.8		
Mar. 9			4.92	47		

*Biomass, nmol P/g media; #AOC concentration, µg eq C/L.

Table 27 Biomass (nmol P/g media) and AOC removal of the filters from March 24 to April 1, 1993

Time	Sample					
	F3		F4		F5	
	Biomass*	ΔAOC#	Biomass*	ΔAOC#	Biomass*	ΔAOC#
Mar. 24	1.17	3	2.18	4.1	1.07	-0.2
Mar. 26			6.61	31		
Mar. 29						
Apr. 1			12.85	-4		

*Biomass, nmol P/g media; #AOC concentration, µg eq C/L.

Table 28 Biomass (nmol P/g media) and AOC removal in the contactors

Time	Sample							
	C2		C3		C4		C5	
	Biomass *	ΔAOC#	Biomass	ΔAOC	Biomass	ΔAOC	Biomass	ΔAOC
Mar. 3	5.47	18			3.62	5.1		
Mar. 9	7.25	44			5.05	5.1		
Mar. 24	3.62	-0.2	0.68	7.0	1.42	2.4	1.84	-2.1
Mar. 26	18.38	85			6.19	44		
Mar. 29	14.15	22						
Apr. 1	6.71	20			4.74	11		

*Biomass, nmol P/g media; #AOC concentration, μg eq C/L.

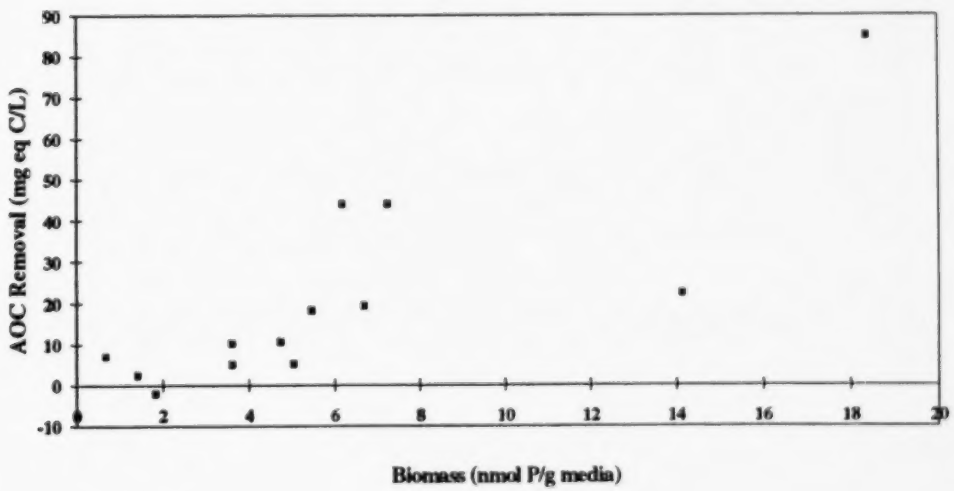


Figure 20 Correlation between AOC removal and biomass (nmol P/g media) in the GAC contactors

6.3.1.2.1. Correlation Between AOC Removal and Biomass (mmol P/m^3 reactor)

For the filters, the same data analysis was conducted by using nmol P biomass/cm^3 reactor instead of nmol P/g media. Because the percentage of anthracite and sand of the samples collected from port 2 of each filter was unknown, the average biomass expressed as nmol P/cm^3 was only the average of ports 0 and 3 instead of the average of ports 0, 2 and 3 used in the analysis with biomass expressed as nmol P/g media. The results were basically the same as for the analysis using nmol P/g media, and no additional insights were obtained. This analysis was not performed for the GAC contactors because the pattern of the results would have remained unchanged.

6.3.1.3 Lag Period Between Change in AOC Concentration and Biomass

During the spring runoff period, when AOC concentrations were changing rapidly, it is reasonable to expect a lag between a change in AOC and a change in biomass. Examination of the data showed that, when the AOC concentration increased, the biomass was not increased immediately but increased at the next sampling. The lag period could be one or two days, but the smallest sampling interval in this study was three days. For example, in Table 29, the AOC concentration on March 26 had increased by a factor of eight from March 24, but the biomass in the filter had only increased by a factor of three. (A greater increase was seen in the GAC contactor.) Although the AOC concentration had dropped on March 29, the filter biomass was much higher. The fact that no further increase occurred in the GAC contactor may have been because substrate was adsorbed.

Because the biomass at a certain depth of a filter or contactor on a certain sampling day was compared to the biomass at the same depth of the same filter or contactor on another sampling day, the results would be the same no matter what units the

biomass was expressed in, so, only the results obtained by using nmol P biomass/g media have been presented here.

Table 29 the AOC influent concentration of Filter 2 and GAC 2 and the biomass at the top layer of Filter 2 and GAC 2.

Time	Sample			
	AOC#	Biomass (F2)	AOC#	Biomass(C2)
Mar. 24	10	2.44	12	4.98
Mar. 26	83	7.66	92	25.84
Mar. 29	25	37.66	24	26.05

*Biomass, nmol P/g media; #AOC concentration, µg eq C/L.

6.3.2 Discussion

The biomass in the GAC contactors was found to be linearly related to the AOC influent concentration and the AOC removal was linearly correlated with the biomass, too. This suggested that the bacterial activity was due to the biodegradable organics and also, that the organic removal was due to the biological activity present in the contactors.

In the dual media filters, no linear relationship could be found between the biomass and the AOC input or AOC removal. This lack of a relationship could be caused by the daily backwashing of the filters, which was shown to decrease the biomass significantly. The biomass measured may therefore not have been able to achieve its steady-state compared to the biofilm formed in the GAC contactors which were backwashed only every week. Any change in the biofilm's environment in addition to AOC concentration still could cause a serious change in the biofilm, so, it appears that the biomass change in the filters was controlled by factors other than the AOC concentration.

Servais et al (1991) found that bacterial activity was responsible for BDOC removal in the filters they studied, and found a fairly good correlation (r=0.7) between bacterial production and BDOC removal. Hijnen and van der Kooij (1992) found a linear relationship between the colony count of the sand at the filter bed surface and the acetate concentration in the influent of the filter.

A lag period between a change in AOC concentration and a change in biomass was observed in the dual media filters but not in the GAC contactors. This lag period could be due to the time required for growth by the bacteria, and the time required for the organisms to acclimate to their new environment and begin to divide.

As stated in the previous section, GAC may be a better medium for biofilm than sand and anthracite. Enrichment of substrate in the biofilm, extended adsorption resulting from bioregeneration, and development of a biofilm that can degrade less biodegradable but adsorbable organics have been cited as reasons why GAC is better than anthracite or sand. The enrichment of substrate and the extended adsorption occurring on GAC may reduce the lag time required by bacteria to acclimate to their new environment and begin to divide since the high organic concentration is more suitable for bacteria to reproduce. The sampling interval was three days, which may be longer than the lag time between an AOC increase and a biomass increase on the GAC.

6.4 Thickness of the Biofilm

The thickness of the biofilm attached to sand, anthracite and GAC was estimated from SEM photographs, in the period from March to April 1993, at the same time as the biomass of the biofilm was measured. The measuring bar at the bottom of each photo was used directly to estimate the thickness of the biofilm. If the biofilm was uniform, the average thickness was estimated using the measuring bar was the thickness of the biofilm, but if the biofilm was patchy, the average thickness was equal to the thickness estimated

using the measuring bar multiplied by the percentage coverage of the biofilm estimated from the SEM photo. This procedure has its limitations because a cross-section view of the particle was not be obtained by using SEM. The distance shown in the bar may not be completely accurate in estimating distance not in the plane of the photo. Therefore the thickness must be regarded as estimates. However these are valid for making relative comparisons and are sufficiently accurate to assist in modeling efforts.

Figure 21 is a selection of SEM photographs; other SEM photographs and their descriptions are presented in Appendix A. Figure 21 A shows the almost uniform biofilm on F2-2 on March 29, 1993; B shows the high magnification of A; C shows the uniform biofilm on C2-0 on April 1, 1993; D shows the high magnification of C; E shows the patchy biofilm on F2-0 on March 26, 1993; F shows the high magnification of E; G shows the patchy biofilm on F3-0 on March 18, 1993 before backwashing; H shows the high magnification of G.

The SEM photos were reviewed by an expert in using SEM to examine biofilm (Sandra Blenkinsopp, Environment Canada, Edmonton). She confirmed that the material covering the particles was in fact biofilm.

Because the amount of biofilm at the bottom layer of the filters was found to be nearly zero in the first SEM examination of the biofilm and biomass measurements, and because the measurements of biomass in the later stages of this study confirmed this result, SEM was only used to observe the biofilm collected from the top and middle layer of each filter.

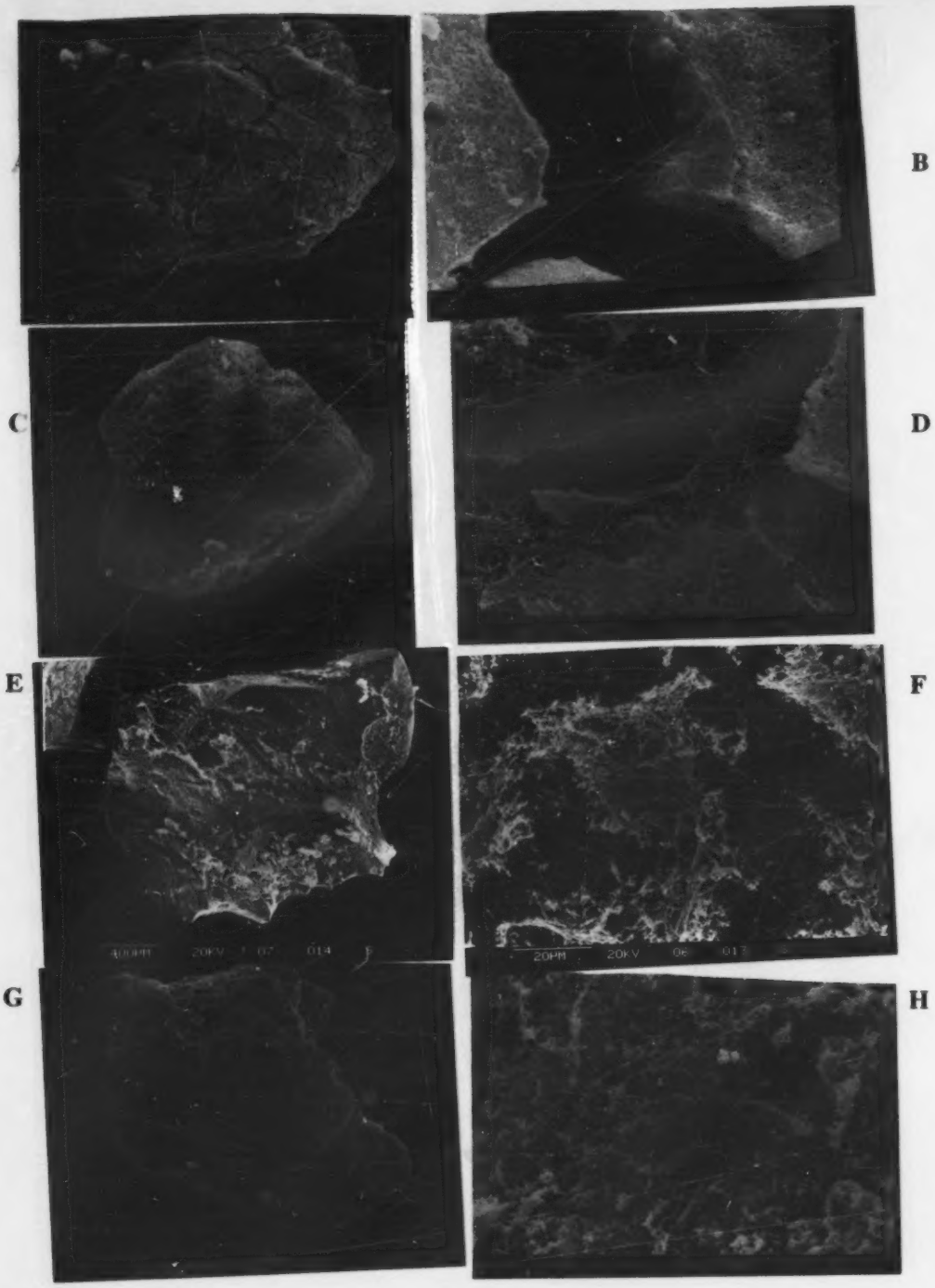


Figure 21 A selection of SEM photographs

6.4.1 Results

In this study, two types of biofilm have been observed by SEM: a uniform biofilm and a patchy biofilm. The uniform biofilm covered the entire surface of the supporting

media, and ranged from very thin (4 µm) to very thick (60 µm) (Figure 21 photos A to D). However, the uniform biofilm was observed mainly on samples collected from the port 0 of GAC contactors.

The patchy biofilm attached to sand or anthracite was mainly in the crevices and cracks area of the surface (Figures 21 photos E to F), but the patchy biofilm on GAC particles was not in the micropores (Figures 21 photos G to H).

From the SEM photos, much more biomass appeared to be present after backwashing in the GAC contactors than in the filters, although this is not necessarily indicated in the biomass measurements in Figure 17. For all filters and contactors it was not possible to establish quantitatively from the photos the effect of backwashing. However the photos do support the general loss of biomass on backwashing seen in Figure 17.

6.4.1.1 Correlation Between Thickness and Biomass (nmol P/g media) of the Biofilm

Tables 30 and 31 give the thickness and the biomass (expressed as nmol P/g media) of the biofilm in the filters and contactors.

Table 30 Thickness and biomass (presented as nmol P/g media) of biofilm in the filters

Sample	Mar. 3		Mar. 10		Mar. 18b		Mar. 18a		Mar. 26		Mar. 29		Apr. 1	
	L#	M_s*	L	M_s	L	M_s	L	M_s	L	M_s	L	M_s	L	M_s
F1-0			0.2	4.66	0.5	1.59	0.5	1.62					0	5.61
F1-2			0	5.88	0.625	4.56	0.1	1.6						6.68
F1-3				9.93		4.56		1.37		5.69				1.92
F2-0	0.15	1.31	0.5	5.39	0	6.57	0.9	4.4	0.25	7.66			1	6.9
F2-2	0.1	2.83	0.2	6.38	0	6.11	0.25	2.12	0	7	60	27.71	0.5	3.35
F2-3	0.25	2.51		4.57		5.28		1.13		3.73		0.83		1.83
F3-0	0.6	4.37	0.2	5.44	0.1	5.3	0.5	4.47	0.5	6.36				
F3-2	0.2	3.58	0	3.88	0	4.59	0.25	4.52		5.44				
F3-3	0.2	3.41		1.45		2.93		2.61		4.79				
F4-0	0.5	6.92	0.5	6.62	10	7.25	0.1	5.11	4	18.3			10	26.95
F4-2	0.25	2.24	1	5.24	5	3.22	0	2.72	1	4.39				7.31
F4-3	0.4	1.74		2.92		0.42				3.21				4.28
F5-0	1.2	2.73	0	4.32	2	4.61	0.25	2.96	4	16.7				
F5-2	0.5	0.8	0	4.12	0	4.38	0	3.9	0	4.49				

F5-3	0.2	0.59		3.83		2.55		2.88		1.47				

#L is the thickness of the biofilm, μm; *M_S is the biomass of the biofilm, nmol P/g media; b: before backwashing; a: after backwashing

Table 31 Thickness and biomass (presented as nmol P/g media) of biofilm in the GAC contactors

Sample	Mar. 3		Mar. 10		Mar. 18b		Mar. 18a		Mar. 26		Mar. 29		Apr. 1	
	L#	M_S*	L	M_S	L	M_S	L	M_S	L	M_S	L	M_S	L	M_S
C2-0			2	12			0.1	3.7			16	26.05	1	8.36
C2-1'											0.1	2.94		
C2-2				2.53	5	4.32		1.44	4	10.9		2.24		5.05
C3-0	0	6.18			0	4.97	0	2.9						
C3-1	0	5.56	0	3.88		2.42		1.58	0	23.6				
C3-2				3.24				2.07		10.8				
C4-0	5	3.95	2.5	5.79		3.85		3.18	0.4	9.76				6.08
C4-2	5	3.29		4.3	5	1.45		1.22	0	2.62				3.4
C5-0	1.6	0.59	10	8.2	2	3.71		3.6	0.5	3.65				
C5-1			2	6.32		2.52		2.03	0	3.16				
C5-2	0	1.42	2	5.21		1.92		0.53		2.76				

#L is the thickness of the biofilm, μm; *M_S is the biomass of the biofilm, nmol P/g media; b: before backwashing; a: after backwashing

By comparing the thickness to the corresponding biomass of the biofilm, generally, the thickness matched well with the biomass of the biofilm, i.e. the SEM photographs provided a good indicator of the amount of biomass present. When a higher biomass was obtained, the thickness was greater: for example, on March 18, the biomass of F4-0 before backwashing was 7.25 nmol P/g media which was higher than the biomass of F4-2 (3.22 nmol P/g media); the thickness of F4-0 was 10 μm which was greater than the thickness of F4-2 (5 μm).

That the thickness measured by SEM was a good indicator of the biomass was confirmed by linear regression analysis. In Figure 22, the thickness of the biofilm in the three ozonated filters was plotted against the biomass of the biofilm, and a good correlation was obtained ($r=0.73$, $r_{table}=0.349$ at $\alpha=5\%$, $\nu=34$). The thickness of the biofilm in all dual media filters was also plotted against the biomass of the biofilm, and again a good correlation was obtained ($r=0.70$, $r_{table}=0.256$ at $\alpha=5\%$, $\nu=52$, Figure not shown). In Figure 23, the thickness of the biofilm in the three ozonated GAC contactors was plotted against the biomass of the biofilm, and a good correlation was obtained ($r=0.71$, $r_{table}=0.468$ at $\alpha=5\%$, $\nu=16$). The thickness of the biofilm in all GAC contactors was plotted against the biomass of the biofilm, and a good correlation was also obtained ($r=0.74$, $r_{table}=0.388$ at $\alpha=5\%$, $\nu=24$, Figure not shown). Therefore, the thickness estimated from SEM photos was a good qualitative indicator for the biomass of the biofilm in any of the streams.

In Figure 22, five apparent outliers were observed. In order to informally test the impact of these apparent outliers and see whether the remaining data exhibited any relationship, these five values were omitted and the correlation between thickness and

biomass for the remaining data determined. This correlation was not significant at the 5% level.

The peeling of the biofilm noted in some photographs, may have hampered the correlation, if it occurred during preparation for SEM examination.

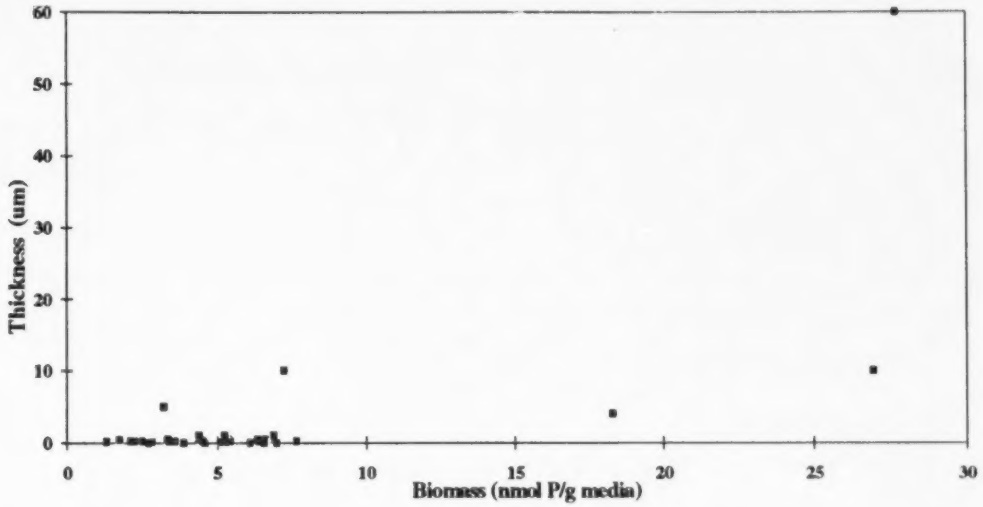


Figure 22 The correlation between the biomass (nmol P/g media) and the thickness of the biofilm in filters 2, 3, 4.

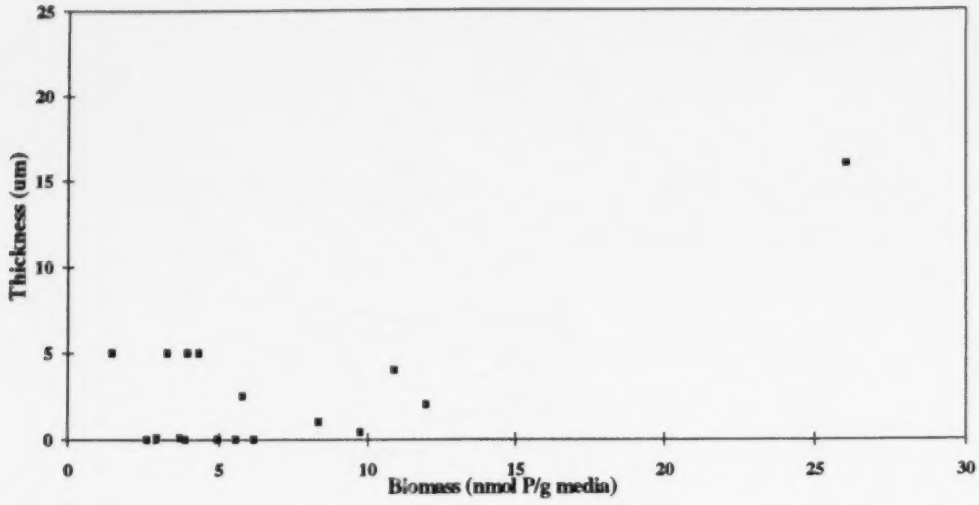


Figure 23 The correlation between the biomass (nmol P/g media) and the thickness of the biofilm in contactors 2, 3 and 4

6.4.1.2 Correlation Between Thickness and Biomass (nmol P/cm^2) of the Biofilm

The thickness of the biofilm will depend on the amount of biomass and the surface area of the media, therefore, biomass was expressed as nmol P/cm^2 by multiplying nmol P/cm^3 by the diameter of the media, which was assumed to be spherical for this calculation. Because the volume of a spherical particle equals 1/6 x π x d^3 (d is the diameter of the particle), the surface area of a spherical particle equals to π x d^2, so, the surface area of particles could be obtained by multiplying the volume by 6 times the diameter of the particle. In examining the correlation between the thickness and nmol P biomass/cm^2, the 6 is a constant which would not impact the result, so, the biomass in nmol P/cm^3 reactor was multiplied by the diameter of the media to obtain a result in nmol P/cm^2.

Tables 32 and 33 show the thickness and the biomass (presented as nmol P/cm^2) of the biofilm in the filters and contactors.

In Figure 24, the thickness of the biofilm in the three ozonated filters was plotted against the biomass of the biofilm, but no significant correlation was obtained ($r=0.24$, $r_{table}=0.444$ at $\alpha=5\%$, $\nu=18$). The thickness of the biofilm in all dual media filters was plotted against the biomass of the biofilm, and again no significant correlation was obtained ($r=0.31$, $r_{table}=0.361$ at $\alpha=5\%$, $\nu=28$, Figure not shown). In Figure 25, the thickness of the biofilm in the three ozonated GAC contactors was plotted against the biomass of the biofilm, and a good correlation was obtained ($r=0.68$, $r_{table}=0.456$ at $\alpha=5\%$, $\nu=17$). The thickness of the biofilm in all GAC contactors was plotted against the biomass of the biofilm, and again a good correlation was obtained ($r=0.63$, $r_{table}=0.388$ at $\alpha=5\%$, $\nu=24$, Figure not shown). Therefore, the thickness estimated from the SEM photos was a good indicator of the biomass of biofilm in either the preozonated or non-ozonated GAC contactors, but not of the biomass of the biofilm in the filters.

Table 32 Thickness and the Biomass (presented as nmol P/cm^2) in the Filters

Sample	Mar. 3		Mar. 10		Mar. 18b		Mar. 18a		Mar. 26		Mar. 29		Apr. 1	
	L	Ms	L	M_s	L	M_s	L	M_s	L	M_s	L	M_s	L	M_s
F1-0		0	0.2	0.46	0.5	0.16	0.5	0.1		0.64		0	0	0.55
F1-3		0		0.91		0.42		0.13		0.52		0		0.18
F2-0	0.15	0.13	0.5	0.53	0	0.64	0.9	0.24	0.25	0.75		3.69	1	0.68
F2-3	0.25	0.23		0.42		0.49		0.14		0.34		0.08		0.17
F3-0	0.6	0.43	0.2	0.53	0.1	0.52	0.5	0.16	0.5	0.62		0		0
F3-3	0.2	0.31		0.13		0.27		0.11		0.44		0		0
F4-0	0.5	0.23	0.5	0.22	10	0.24	0.1	0.11	4	0.4		0	10	0.89
F4-3	0.4	0.16		0.27		0.04		0.11		0.3		0		0.39
F5-0	1.2	0.27	0	0.42	2	0.45	0.25	0.23	4	1.64		0		0
F5-3	0.2	0.05		0.35		0.23		0.04		0.14		0		0

L is the thickness of the biofilm, μm; M_S the biomass of the biofilm, nmol P/cm^2.

Table 33 Thickness and the Biomass (presented as nmol P/cm^2) in the GAC contactors

Sample	Mar. 3		Mar. 10		Mar. 18b		Mar. 18a		Mar. 26		Mar. 29		Apr. 1	
	L	M_s	L	M_s	L	M_s	L	M_s	L	M_s	L	M_s	L	M_s
C2-0		0.21	2	0.29		0.31	0.1	0.12		0.63	16	0.64	1	0.2
C2-2		0.14		0.16	5	0.28		0.15	4	0.7		0.14		0.33
C3-0	0	0.15		0.12	0	0.12	0	0.02		0.66		0		0
C3-1	0	0.2	0	0.14		0.09		0.03	10	0.85		0		0
C3-2		0.06		0.21		0.06		0.03		0.7		0		0
C4-0	5	0.1	2.5	0.14		0.09		0.03	4	0.24		0		0.15
C4-2	5	0.21	0.2	0.28	5	0.09	NA	!	4	0.17		0		0.22
C5-0	1.6	0.01	10	0.2	2	0.09	NA	0.07	0	0.09		0		0
C5-1		0.14	2	0.23		0.09		0.03		0.11		0		0
C5-2	0	0.09	2	0.34		0.12		0.06		0.18		0		0

L is the thickness of the biofilm, μm; M_S the biomass of the biofilm, nmol P/cm^2.

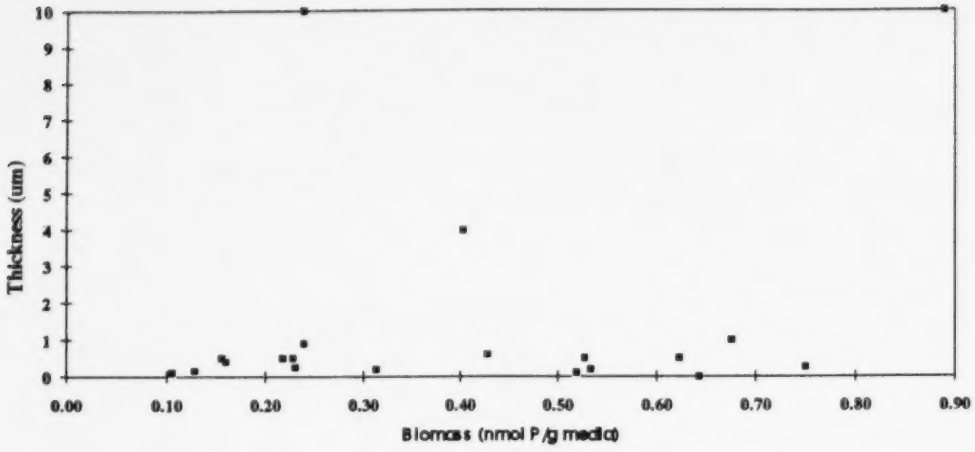


Figure 24 The correlation between the thickness and the biomass (nmol P/cm^2) of the biofilm in Filters 2, 3 and 4

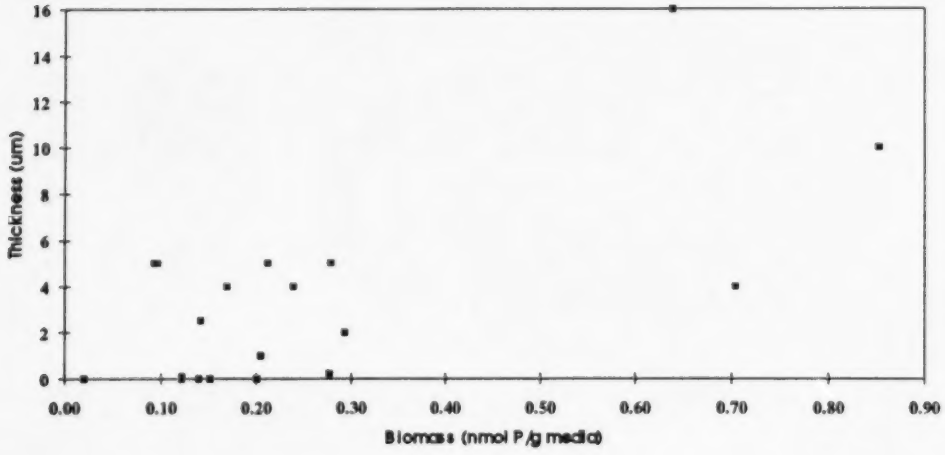


Figure 25 The correlation between the thickness and the biomass (nmol P/cm^2) of the biofilm in contactors 2, 3 and 4

6.4.2 Discussion

Although SEM combined with freeze dry preparation of the sample was found to cause less shrinkage than the SEM combined with critical point drying in the preliminary

evaluation of the methods, but shrinkage of the biofilm was still observed in this study. When the biomass of the biofilm was not zero, the thickness was found frequently to be zero. This phenomenon showed that there was shrinkage of the biofilm caused by preparation of the sample for the SEM observation. Therefore, the thickness of the biofilm estimated by SEM (freeze drying) could be used as an indicator of the biomass of the biofilm, but it is not a very accurate value. For biomass expressed as nmol P/g media, reasonable correlations were obtained for all bioreactors. For biomass in nmol P/cm^2, a reasonable correlation was only obtained for the GAC contactors.

A uniform biofilm is one of the several assumptions of mathematical models for describing substrate utilization by a biofilm. In this study, a uniform biofilm was observed several times by SEM, which provided visual evidence for the assumption of the mathematical model. However, the uniform biofilm was only observed on samples which were collected from the top layer of the dual media filters or GAC contactors. It was suspected that this was due to the fact that the biomass decreased with the depth of the filter or GAC contactor. By studying four sand filters, Hijnen and van der Kooij (1992) found that microbiological activity was high in the top layer of the filters and influence the concentration of bacteria deeper in the filter bed. Servais et al. (1992) found that the biomass decreased significantly from the top to the bottom of the GAC contactors at the Choisy-le-Roi treatment plant, near Paris, France.

A patchy biofilm has been observed in water treatment plants by other researchers (Bouillot et al., 1992, Cairo et al., 1979, Weber et al., 1978, LeChevallier et al., 1984).

6.5 Effect of Biofilm on HPC

HPC counts were measured using R2A agar, incubating at 22^{o}C for 7 days. The HPC in the influent and effluent of each filter and contactor were measured on each

sampling day during the period from March to April 1993. The HPC in the influent and effluent water of the filters and contactors are listed in Tables 34 and 35.

6.5.1 Results

From Table 36, the HPC in the effluent of the ozonated filters was found to be consistently substantially higher than the HPC in the influent. For the control filters (F1 and F5) HPC in the effluent were the same or lower.

From Table .7, the HPC in the effluent of the contactors was sometimes higher than in the influent, but sometimes lower. No significant difference was found by a paired t-test ($\alpha = 0.166$).

Linear regression analysis showed that the HPC increase or the log of the effluent HPC were not linearly related to the biomass (average biomass) present in the filters or contactors.

6.5.2 Discussion

There could be three reasons for the fact that the HPC in the effluent of the ozonated filters was higher than the HPC in the influent, but this phenomenon was not present in the GAC contactors: 1. the daily backwashing of the filters, 2. the disinfection effect caused by preozonation which killed most of the HPC organisms present in the influent. The lack of an increase through the GAC suggests that the biomass in the GAC was relatively small.

Servais et al. (1991) found that bacteria seemed to be more numerous in the outflow than in the inflow of a GAC filter which was washed every 50 h to 100 h in the Choisy-le-Roi plant. This indicated a small net exportation of bacterial biomass from the filter into the outflow. Therefore, the advantage offered by GAC was not the reason for

the phenomenon that the HPC in the effluent was not more than the HPC in the influent in the current study.

The dual media filters in this study were backwashed every day, whereas the GAC contactors were backwashed every week, so the biofilm attached to the filter media may not have been at steady state, bacteria attached to the filter media may not have been more easily washed off than the bacteria attached to the GAC particles. This may be one of the reasons why the HPC in the effluent of the filters were higher than the influent but this phenomenon was not found in the GAC contactors.

Table 34 The HPC of the influent and effluent of each filter

Date (1993)	Sample (CFU/mL)									
	F1		F2		F3		F4		F5	
	Infl	Effl	Infl	Effl	Infl	Effl	Infl	Effl	Infl	Effl
Mar. 3	30	10	10	11000			10	10000		
Mar. 9	30	10	60	8600			60	4200		
Mar. 24	120	30	140	3000	140	3000	140	1600	13000	7800
Mar. 26	30	10	3200	11000			3200	7000		
Mar. 29			110	5700						
Apr. 1	10	10	80	5300			80	7800		

Table 35 The HPC of the influent and effluent of each GAC contactor

Date (1993)	Sample (CFU/mL)							
	C2		C3		C4		C5	
	Infl	Effl	Infl	Effl	Infl	Effl	Infl	Effl
Mar. 3	11000	8800			11000	12000		
Mar. 9	8600	8700			4200	8000		
Mar. 24	3000	1400	3000	3000	1600	2400	7800	1300
Mar. 26	11000	7700			7000	8500		
Mar. 29	5700	13000						
Apr. 1	5300	300000			7800	9700		

6.6 Overall Discussion

Similar research work has been conducted by Miltner et al. (1993). Biomass was measured in the period October 25 to November 22, 1991, using the same method as in the present study. Much more biomass was found in the study conducted by Miltner et al. than in the present study. Generally, the biomass in this study was in the range of 0.43 to 37.66 nmol P/g media, but in the study conducted by Miltner et al., the biomass was in the range of 2 to 470 nmol P/g media. This phenomenon may be caused by the temperature difference and the treatment difference between these two studies. For example, in the study conducted by Miltner et al., the temperature only fell below 17^{o}C in the settled water, which was the influent to the filters, after day 93. In the present study, the temperature after filtration never was above 13^{o}C (Appendix C). Also, there were some differences in the treatment process between these two studies. In the study conducted by Miltner et al. ozone was added before sedimentation, whereas in this study, the ozone was added after sedimentation. Probably due to these and other factor, a higher AOC concentration was found in the study conducted by Miltner et al. than in the present study.

All of these reasons would explain why a higher amount of biomass was found by Miltner et al. than in this study.

An increase in AOC-P17 during biofiltration was found by Miltner et al., too. Two hypotheses for this were offered by these authors. In the first, oxalate-type nutrients preferred by the *Spirillum* stain NOX were being biodegraded and acetate-type nutrients preferred by the *pseudomonas* stain P17 were being produced. It may be that this AOC-P17 production in biological filters is more dramatic in waters that have been previously ozonated, resulting in AOC-NOX being a relatively higher percentage of the total AOC, than in biological filters not previously ozonated. Waters relatively high in AOC-NOX may promote a population of acclimated bacteria that produce acetate-type compounds preferred by the P17 strain. If this hypothesis is true, an important question is whether increases in AOC-P17 during biological filtration have any impact on microbial regrowth in the distribution system.

In the second hypothesis, the increase in AOC-P17 may only be an apparent increase as a result of the manner in which the AOC test was conducted. Standard Methods (1990) was followed and P17 and NOX strains were introduced simultaneously. Total AOC is defined as the sum of P17 and NOX growth yields. This includes compounds that can be assimilated by both strains. If the P17 and NOX are introduced simultaneously during the test, competition between P17 and NOX occurs for available nutrients including the common AOC that can be utilized by both.

In settled water, oxalate and other ozone byproduct compounds are present as a result of ozonation. These compounds allow the NOX to compete better for available nutrients. Thus, NOX can dominate in the test and outcompete P17 for common compounds resulting in an AOC-P17 concentration that might be lower than if the NOX strain was not present during the test.

In biofiltered water, bacteria have degraded ozone byproducts as they have acclimated to them. Since these compounds are biodegraded, less substrate is available for NOX. Thus, in the AOC test, NOX cannot compete as well as when the ozone byproducts were present, and the P17 is able to use more of the common substrate. As a result, even though the total AOC and each fraction may have decreased during biofiltration, the AOC-P17 concentration appears to increase because the P17 was able to better compete during the test.

Van der Kooij et al. (1987) report decreases in the concentrations of both AOC-NOX and AOC-P17 during biological filtration in two treatment plants, but the P17 and NOX strains were inoculated separately in the test.

If the second hypothesis by Miltner et al. (1993) is true, separate or simultaneous inoculation of the P17 and NOX strains in the AOC test must be considered in the experimental design of a project so the resulting data can be used to fairly evaluate various treatment processes.

Miltner et al. (1993) also found that the biomass decreased immediately after backwashing the filters.

A conversion factor between the phospholipid and the cell number in soil/sediment has been found by Balkwill et al. (1988). 1 g dry wt cells = 50 μmol P, 1 g dry wt cells =2 x 10^{13} cells, so, 1 μmol P = 4 x 10^{11} cells, 1 nmol P = 4 x 10^{8} cells. Tables 36, 37 and 38 are the biomass in units of nmol P/g media and number of cells/g media in the filters and contactors from February 25 to April 1, 1993.

By using conversion factor (1 nmol P = 4 x 10^{8} cells), the biomass in this study was found to range from 1.72 x 10^{8} to 151 x 10^{8} cells/g media. Biomass (expressed as no. cells/g media) in the dual media filters was 10^{5} to 10^{7} times the HPC level in the

influent of filter which ranged from 10 to 3200 CFU/mL, but the biomass in the GAC contactors was only 10^4 to 10^5 times the HPC level in the influent of the contactor which ranged from 1600 to 11000 CFU/mL. Therefore, the biomass increase in the dual media filters was much more than in the GAC contactors compared to their influent HPC level. This fact may be one of the reasons why HPC levels increased after biological dual media filters but not in the GAC contactors.

Table 36 The biomass in unit of nmol P/g media and number of cells/g media from February 25 to March 10, 1993

Sample	Feb. 25		Mar. 3		Mar. 10	
	nmol/g media	10^8cells/g media	nmol/g media	10^8cells/g media	nmol/g media	10^8cells/g media
F1-0	2.61	10.4			4.66	18.6
F1-2	5.05	20.2			5.88	23.5
F1-3	1.36	5.44			9.93	39.7
F2-0	5.27	21.1	1.31	5.24	5.39	21.6
F2-2	4.02	16.1	2.83	11.3	6.38	25.5
F2-3	4.64	18.6	2.51	10.0	4.57	18.3
F3-0	3.66	14.6	4.37	17.5	5.44	21.8
F3-2	2.65	10.6	3.58	14.3	3.88	15.5
F3-3	2.5	10.0	3.41	13.6	1.45	5.80
F4-0	4.13	16.5	6.92	27.7	6.62	26.5
F4-2	2.63	10.5	2.24	8.96	5.24	21.0
F4-3	1.71	6.84	1.74	6.96	2.92	11.7
F5-0	4.71	18.8	2.73	10.9	4.32	17.3
F5-2	4.18	16.7	0.8	3.20	4.12	16.5
F5-3	2.02	8.08	0.59	2.36	3.83	15.3
C2-0	3.71	14.8	8.76	35.0	11.97	47.9
C2-2	2.83	11.3	2.18	8.72	2.53	10.1
C3-0	5.36	21.4	6.18	24.7	5.07	20.3
C3-1	4.56	18.2	5.56	22.2	3.88	15.5
C3-2	2.86	11.4	0.94	3.76	3.24	13.0
C4-0	7.03	28.1	3.95	15.8	5.79	23.2
C4-2	0.76	3.04	3.29	13.2	4.3	17.2
C5-0	6.21	24.8	0.59	2.36	8.2	32.8
C5-1	2.78	11.1	3.94	15.8	6.32	25.3
C5-2	3.63	14.5	1.42	5.68	5.21	20.8

Table 37 The biomass in unit of nmol P/g media and number of cells/g media in filters from March 18 to April 1, 1993

Sample	Mar. 18#		Mar. 18*		Mar. 24		Mar. 26		Mar. 29		Apr. 1	
	nmol/ g media	10^8ce lls/g media	nmol/ g media	10^8ce lls/g media	nmol/ g media	10^8ce lls/g media	nmol/ g media	10^8ce lls/g media	nmol/ g media	10^8ce lls/g media	nmol/ g media	10^8ce lls/g media
F1-0	1.59	6.36	1.62	6.48	1.05	4.20	6.55	26.2			5.61	22.4
F1-2	4.56	18.2	1.60	6.40	1.75	7	9.65	38.6			6.68	26.7
F1-3	4.56	18.2	1.37	5.48	1.39	5.56	5.69	22.8			1.92	7.68
F2-0	6.57	26.3	4.40	17.6	2.44	9.76	7.66	30.6	37.66	151	6.9	27.6
F2-2	6.11	24.4	2.12	8.48	2.34	9.36	7	28.0	27.71	111	3.35	13.4
F2-3	5.28	21.1	1.13	4.52	1.55	6.20	3.73	14.9	0.83	3.32	1.83	7.32
F3-0	5.3	21.2	4.47	17.9	1.59	6.36	6.36	25.4				
F3-2	4.59	18.4	4.52	18.1	0.75	3.00	5.44	21.8				
F3-3	2.93	11.7	2.61	10.4	1.18	4.72	4.79	19.2				
F4-0	7.25	29.0	5.11	20.4	3.21	12.8	12.22	48.9			26.95	108
F4-2	3.22	12.9	2.72	10.9	NA		4.39	17.6			7.31	29.2
F4-3	0.42	1.68			1.15	4.60	3.21	12.8			4.28	17.1
F5-0	4.61	18.4	2.96	11.8	2.39	9.56	16.73	66.9				
F5-2	4.38	17.5	3.90	15.6	0.4	1.60	4.49	18.0				
F5-3	2.55	10.2	2.88	11.5	0.43	1.72	1.47	5.88				

samples collected on March 18, 1993 just before backwashing, *samples collected on March 18, 1993 immediately after backwashing.

Table 38 The biomass in unit of nmol P/g media and number of cells/g media in contactors from March 18 to April 1, 1993

Sample	Mar. 18#		Mar. 18*		Mar. 24		Mar. 26		Mar. 29		Apr. 1	
	nmol/ g media	10^8ce lls/g media	nmol/ g media	10^8ce lls/g media	nmol/ g media	10^8ce lls/g media	nmol/ g media	10^8ce lls/g media	nmol/ g media	10^8ce lls/g media	nmol/ g media	10^8ce lls/g media
C2-0	12.5	50.0	3.70	14.8	4.98	19.9	25.84	103	26.05	104	8.36	33.4
C2-2	4.32	17.3	1.44	5.76	2.25	9.00	10.92	43.7	2.24	8.96	5.05	20.2
C3-0	4.97	19.9	2.90	11.6	0.84	3.36	27.05	108				
C3-1	2.42	9.68	1.58	6.32	0.89	3.56	23.61	94.4				
C3-2	0.95	3.80	2.07	8.28	0.52	2.08	10.84	43.4				
C4-0	3.85	15.4	3.18	12.7	1.42	5.68	9.76	39.0			6.08	24.3
C4-2	1.45	5.80	1.22	4.88	NA		2.62	10.5			3.4	13.6
C5-0	3.71	14.8	3.60	14.4	2.78	11.1	3.65	14.6				
C5-1	2.52	10.1	2.03	8.12	0.76	3.04	3.16	12.6				
C5-2	1.92	7.68	5.30	2.12	0.9	3.60	2.76	11.0				

Chapter 7 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

7.1 Conclusions

In this study, methods for measuring the thickness and the biomass of the biofilm in a biological water treatment plant were evaluated. A phospholipid determination using malachite green and SEM combined with freeze drying preparation were used to measure the biomass and the thickness of the biofilm respectively. Generally, the phospholipid determination provided a fairly good and sensitive measurement for biomass, and the thickness estimated by SEM provided a good indicator for the biomass.

The results gained during this pilot study of biological treatment have very important consequences for the design and management of biological treatment in drinking water treatment plants.

First of all, the experimental data confirm the usefulness of preozonation to improve the biological treatment by increasing the biomass of the biofilm, higher amount of biofilm was produced in stream 2 which was fed with preozonated water than in stream 5 which was fed with non-preozonated water. More biomass was present in Filter 1 which was fed with prechlorinated water than in Filter 5 which was fed with non-prechlorinated water. This fact showed that prechlorination did not kill all the bacteria in the water and did not prevent those bacteria from attaching to the filter media and forming biofilm. A higher amount of biofilm was produced in Streams 2 than in Stream 1. Biomass in the GAC contactors was linearly related to the AOC input concentration, and the AOC removal in the contactors was linearly correlated with the bacterial activity in the contactors, but these correlations did not occur in the dual media filters.

As far as design is concerned, stream 2 which was operated at higher hydraulic loading (10 m/h) showed more biofilm than stream 3 which was at lower hydraulic loading

(5 m/h). Also, GAC F300 was not a better media for bacteria to form biofilm than anthracite based on nmol P/cm^3, and GAC F400 and Pica carbon were not better media than sand based on nmol P/cm^3. Too deep a filter or contactor is useless because the biomass decreases along the depth of the filter. If the filter or contactor is too deep, there is no biofilm attached at the bottom of the filter or contactor and no biological treatment is performed in that region. The appropriate contactor depth (EBCT for a given hydraulic loading) in a given situation would depend on influent AOC levels and other parameters such as temperature.

As far as management is concerned, backwashing of filters and contactors decrease the biofilm significantly, but backwashing did not change the vertical stratification of biomass in the filters and GAC contactors. In this study, biological treatment increased the HPC in the effluent of the dual media filters but not in the effluent of the GAC contactors.

7.2 Recommendations For Future Work

If thicker biofilm is expected, the thickness of biofilm attached to sand and anthracite could be measured by the moisture method (Rittmann et al., 1986). If the thickness could be estimated by SEM at the same time, the shrinkage factor caused by SEM could be found. Then, any thicknesses that have been estimated by SEM could be multiplied by this shrinkage factor, to provide a more accurate value. However, before the thickness of the biofilm attached on sand or anthracite is measured by the moisture method, it must be established that the biofilm is uniform, because the moisture method assumes a the uniform biofilm.

The biomass of the biofilm attached to sand or anthracite could be measured by the phospholipid determination using the malachite green method and simultaneously by other methods such as standard VSS and its modifications (ATP, carbohydrate, protein, organic

nitrogen), COD and radio-labeled tracers so the conversion factor between these methods could be found. This would facilitate comparisons among studies.

In the present study, if manpower and glassware are available, the frequency of the AOC measurements could be increased to every day during the spring runoff period, so there will be enough data to establish the lag period between change in AOC concentration and biomass. Linear regression analysis could be used to find if the biomass is related to the AOC of one, two or three days previously.

Also, the AOC profile could be measured, so, the amount of biomass which is enough to achieve the desirable AOC removal could be found. However, an arrangement is needed between the person who is responsible for the measurement of biomass and the person who is responsible for the AOC measurements, so, the sample will be collected from the same depth of the filters and contactors.

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Appendix A
SEM PHOTOGRAPHS

Tables 1 to 7 are the detailed description of the biofilm on each sampling day. The photographs referred to are in Appendix A.

Table 1 Description of biofilm collected on March 3, 1993

Sample Source	Description
F2 (Photos 1-9)	Biofilm was patchy in F2, but serious biofilm peeling has happened on F2-0 because the surface of the F2-0 was very clean. The biofilm decreased with the depth of the filter.
F3 (Photos 10-18)	Biofilm was almost uniform on F3-0, but it was patchy on F3-2 and F3-3. The very clean surface of F3-2 showed that a serious biofilm peeling has happened. Biofilm on F3-0 was more than biofilm on F3-3 which was more than the biofilm on F3-2.
F4 (Photos 19-27)	Biofilm was almost uniform on F4-0, but it was patchy on F4-2 and F4-3. Also, the patchy biofilm on F4-2 and F4-3 mainly occurred in the crevice area.
F5 (Photos 28-33)	Biofilm was uniform on F5-0 and F5-2, but it was patchy on F5-3 which may be caused by peeling of the biofilm because the surface of F5-3 was quite clean.
C2 (Photos 34-39)	Biofilm was uniform on C2-2 but it was patchy on C2-0 which may be caused by the peeling of the biofilm.
C3 (Photos 40-46)	Biofilm was patchy on C3-0, C3-1 and C3-2.
C4 (photos 47-50)	Biofilm was uniform on C4-0, and it was almost uniform on C4-2.
C5 (Photos 51-56)	Biofilm was uniform on C5-1, but it was patchy on C5-0 and C5-2. The patchy biofilm on C5-0 may be caused by peeling of the biofilm.

Table 2 Description of biofilm collected on March 10, 1993

Sample Source	Description
F1 (Photos 1-4)	Biofilm was patchy on F1-0, F1-2.
F2 (Photos 5-8)	Biofilm was patchy in F2, the patchy biofilm on F2-2 mainly occurred in the crevice area.
F3 (Photos 9-11)	Biofilm was patchy on F3-0.
F4 (Photos 12-15)	Biofilm was almost uniform on F4-2, but it was patchy on F4-0 which may be caused by the biofilm peeling during sample preparation.
F5 (Photos 16-20)	Biofilm was patchy on F5-0 and F5-2; peeling of biofilm has happened on F5-0 because the surface of F5-0 was quite clean. Also, a very interesting phenomenon happened on F5-0; after the biofilm peeling, individual bacteria were observed on the surface of F5-0 which could not be observed when the biofilm was present. This phenomenon showed that when the biofilm existed, the bacteria were covered under the biofilm.
C2 (Photos 21-25)	Biofilm was uniform on C2-0 and C2-2.
C3 (Photos 26-31)	Biofilm was patchy on C3-0 and C3-2, but the biofilm was not in the pore.
C4 (photos 32-33)	Biofilm was patchy on C4-0.
C5 (Photos 34-37)	Biofilm was almost uniform on C5-0 and C5-2.

Table 3 Description of biofilm collected on March 18, 1993 before backwashing

Sample Source	Description
F1 (Photos 1-4)	Biofilm was patchy on F1-0, F1-2.
F2 (Photos 5-8)	Biofilm was patchy in F2, serious biofilm peeling has happened.
F3 (Photos 9-16)	Biofilm was patchy on F3-0 and F3-2.
F4 (Photos 17-20)	Biofilm was uniform on F4-0, but it was patchy on F4-2.
F5 (Photos 21-24)	Biofilm was patchy on F5-0 and F5-2; peeling of biofilm has happened on F5-0 because the surface of F5-0 was quite clean.
C2 (Photos 25-28)	Biofilm was uniform on C2-0 and C2-2.
C3 (Photos 29-32)	Biofilm was uniform on C3-0 and C3-2; in a few areas of the surface of C3-0 and C3-2, biofilm peeling happened.
C4 (photos 33-36)	Biofilm was patchy on C4-0, but biofilm was uniform on C4-2.

Table 4 Description of biofilm collected on March 18, 1993 after backwashing

Sample Source	Description
F1 (Photos 1-4)	Biofilm was patchy on F1-0, F1-2. Note cracking of the anthracite in Photo 2 (F1-0).
F2 (Photos 5-8)	Biofilm was patchy in F2-0 and F2-2.
F3 (Photos 9-12)	Biofilm was patchy and sparse on F3-0 and F3-2.
F4 (Photos 13-16)	Biofilm was patchy on F4-0 and F4-2, but biofilm peeling occurred on both samples. More biomass may have been present in F4 than in the other filters.
F5 (Photos 17-18)	Biofilm was patchy on F5-0.
C2 (Photos 19-20)	Biofilm was patchy on C2-0.
C3 (Photos 21-22)	Biofilm was patchy on C3-0.
C4 (photos 23-24)	Biofilm was patchy on C4-0.
C5 (Photos 25-26)	Biofilm was patchy on C5-0.

Table 5 Description of biofilm collected on March 26, 1993

Sample Source	Description
F1 (Photos 1-5)	Biofilm was patchy on F1-0, F1-2.
F2 (Photos 6-9)	Biofilm was patchy on F2-0 and F2-2.
F3 (Photos 10-11)	Biofilm was patchy on F3-0.
F4 (Photos 12-15)	Biofilm was almost uniform on F4-0 and almost uniform on F4-2.
F5 (Photos 16-19)	Biofilm was almost uniform on F5-0 and F5-2.
C2 (Photos 20-23)	Biofilm was patchy on C2-0 and biofilm peeling was happened, but the biofilm was almost uniform on C2-2.
C3 (Photos 24-27)	Biofilm was almost uniform on C3-0 and C3-1.
C4 (photos 28-31)	Biofilm was patchy on C4-0 but was almost uniform on C4-2.
C5 (Photos 32-35)	Biofilm was almost uniform on C5-0 and C5-2.

Table 6 Description of biofilm collected on March 29, 1993

Sample Source	Description
F2 (Photos 1-4)	Biofilm was patchy on F2-0 and F2-2, but serious biofilm peeling happened, especially on F2-0.
C2 (Photos 5-9)	Biofilm was uniform on C2-0, but it was patchy on C2-2.

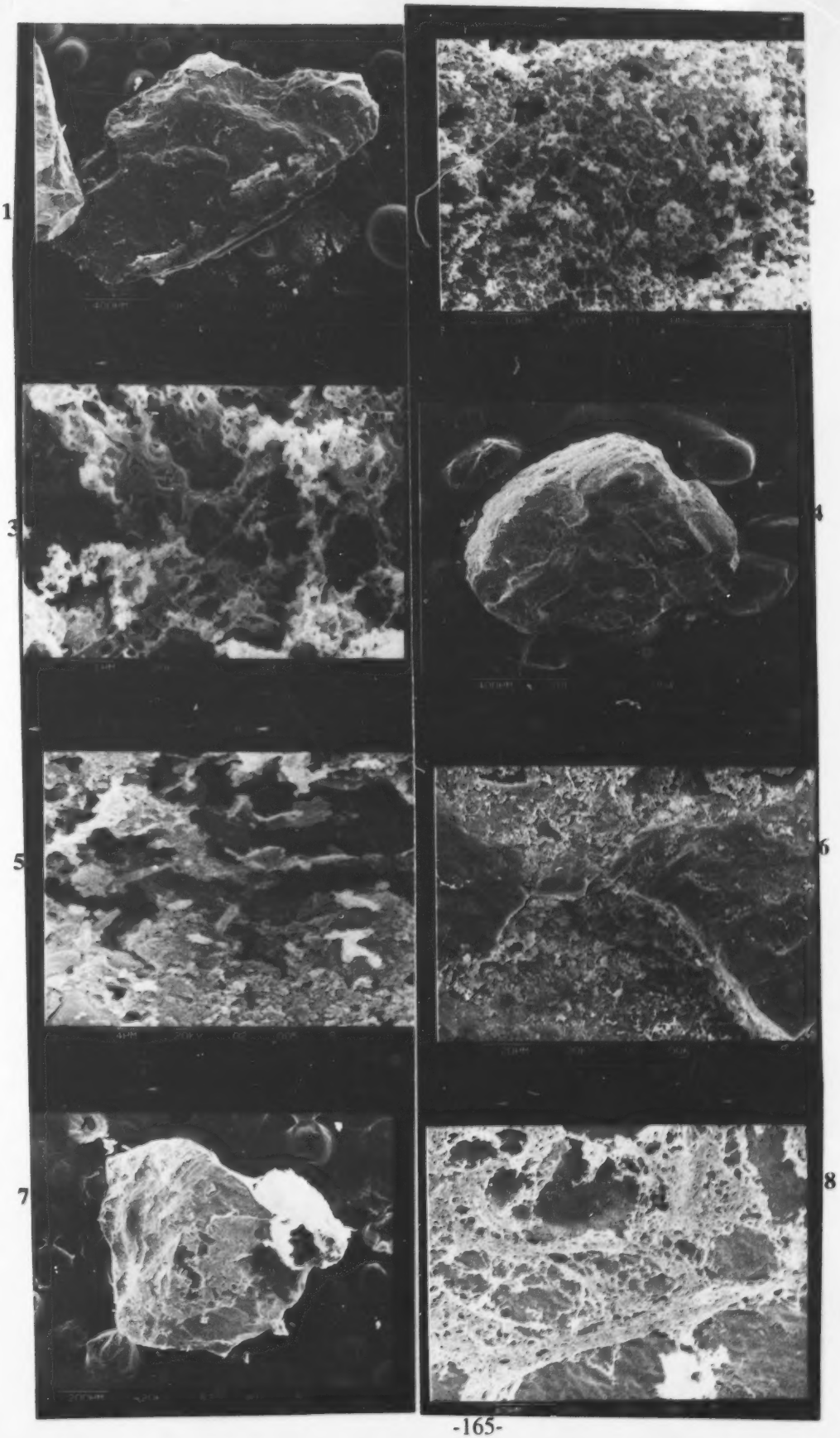
Table 7 Description of biofilm collected on Apr 1, 1993

Sample Source	Description
F1 (Photos 1-3)	Biofilm was patchy on F1-0.
F2 (Photos 4-7)	Biofilm was patchy on F2-0 and F2-2.
C2 (Photos 8-11)	Biofilm was uniform on C2-0, and nearly uniform on C2-2.

SEM PHOTOGRAPHS OF SAMPLES ON MARCH 3, 1993

Number of photo	Biofilm
1	F2-0
2	high manification of 1
3	high manification of 2
4	F2-2
5	high manification of 4
6	high manification of 5
7	F2-3
8	high manification of 7
9	high manification of 8
10	F3-0
11	high manification of 10
12	high manification of 11
13	F3-2
14	high manification of 13
15	high manification of 13
16	F3-3
17	high manification of 16
18	high manification of 17
19	F4-0
20	high manification of 19
21	high manification of 20
22	high manification of 21
23	F4-2
24	high manification of 23
25	high manification of 24
26	F4-3
27	high manification of 26
28	F5-0
29	high manification of 28
30	F5-2
31	high manification of 30
32	F5-3
33	high manification of 32
34	C2-0
35	high manification of 34
36	high manification of 35
37	C2-2
38	high manification of 37
39	high manification of 38
40	C3-0
41	high manification of 40
43	C3-1
44	high manification of 43

45	C3-2
46	high manification of 45
47	C4-0
48	high manification of 47
49	C4-2
50	high manification of 49
51	C5-0
52	high manification of 51
53	C5-1
54	high manification of 53
55	C5-2
56	high manification of 55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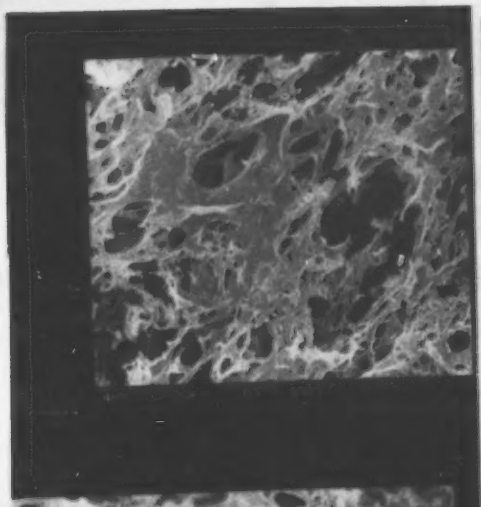

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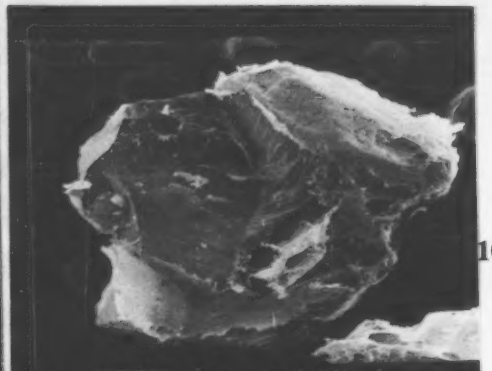

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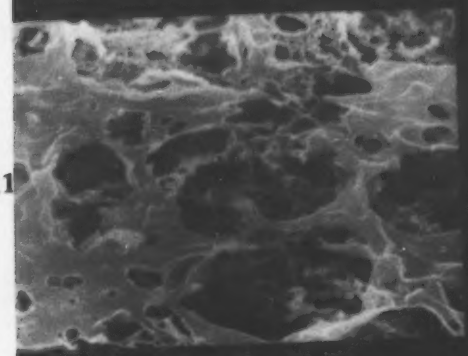

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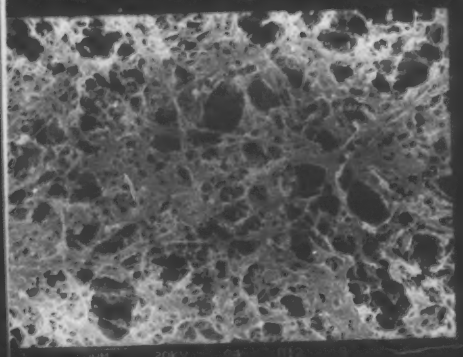

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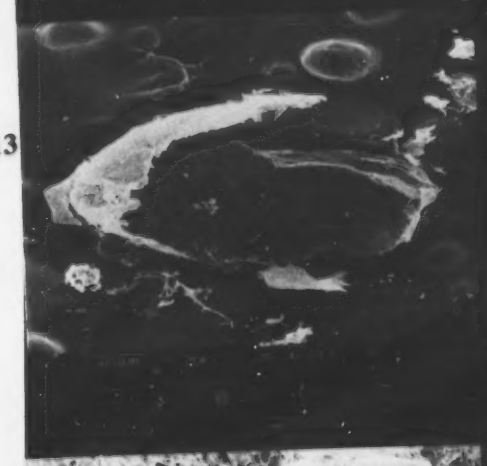

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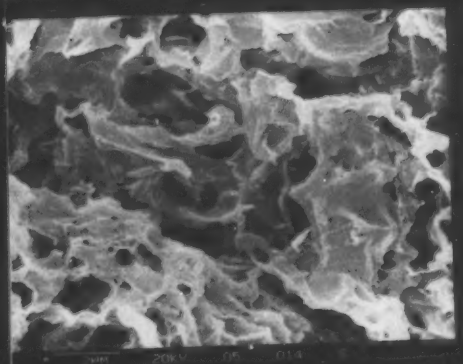

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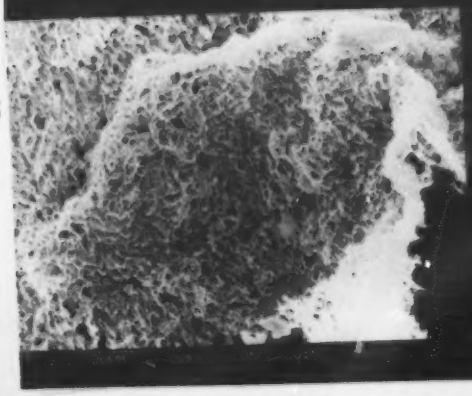

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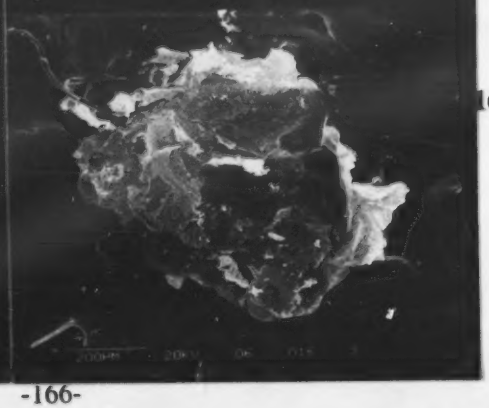

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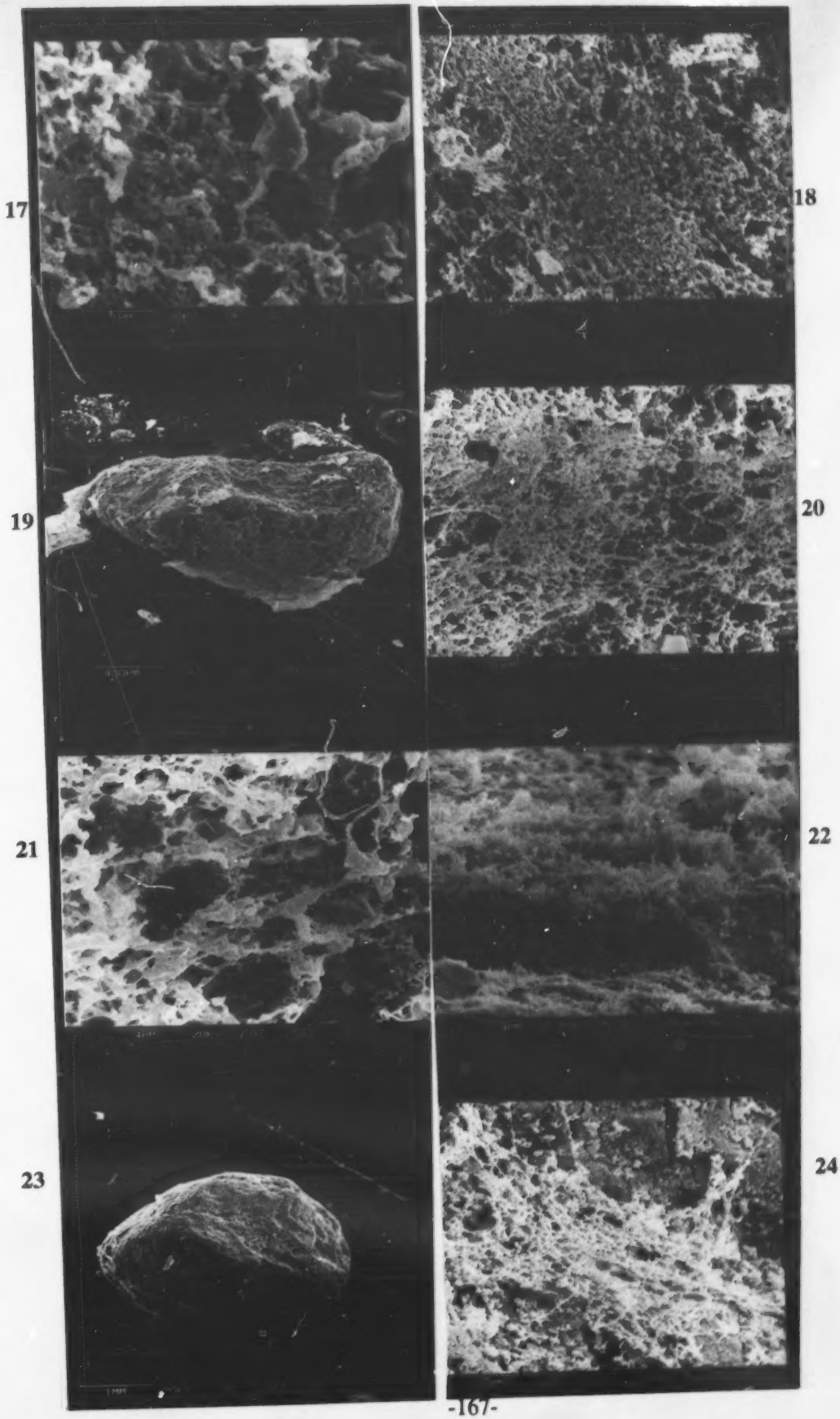

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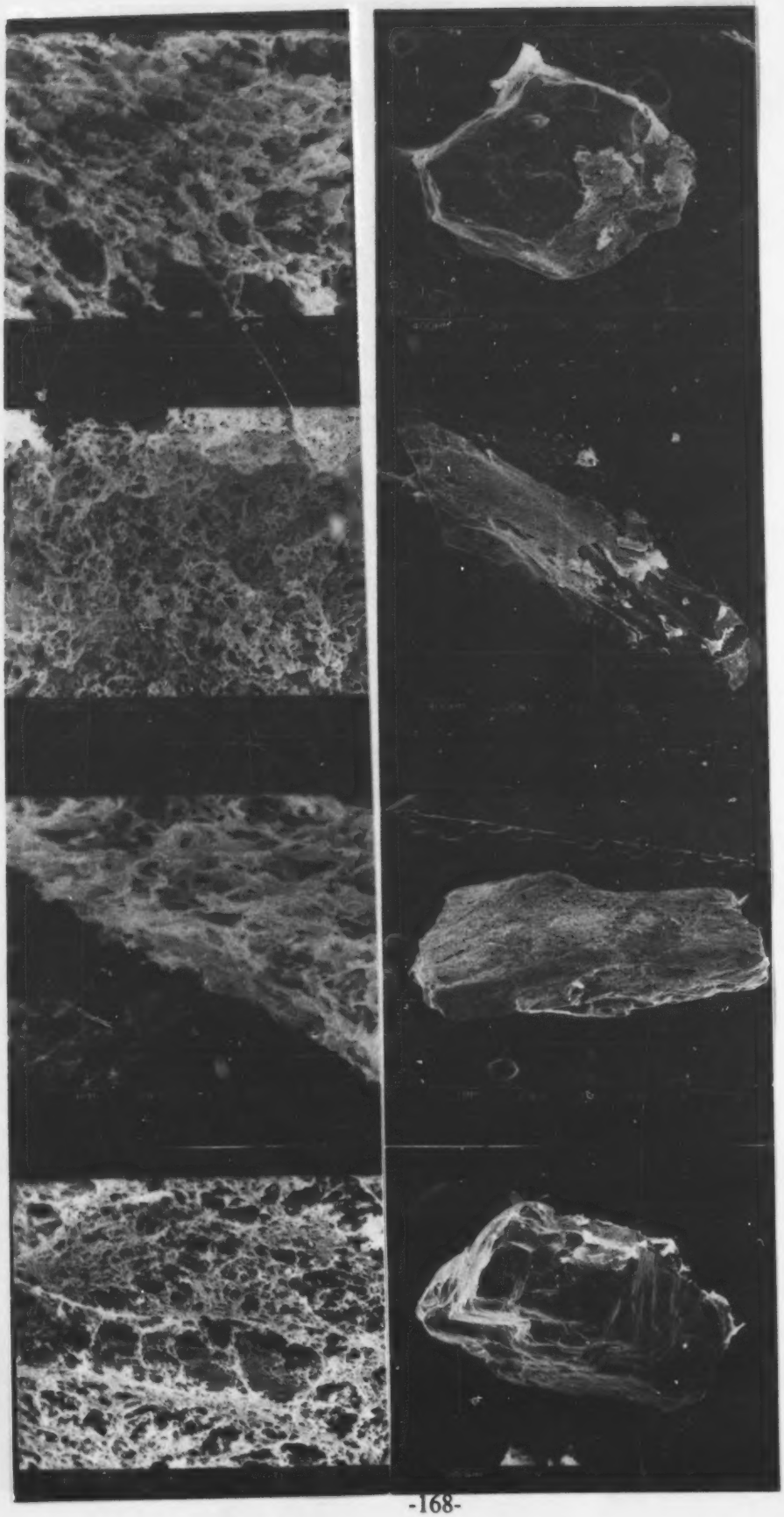

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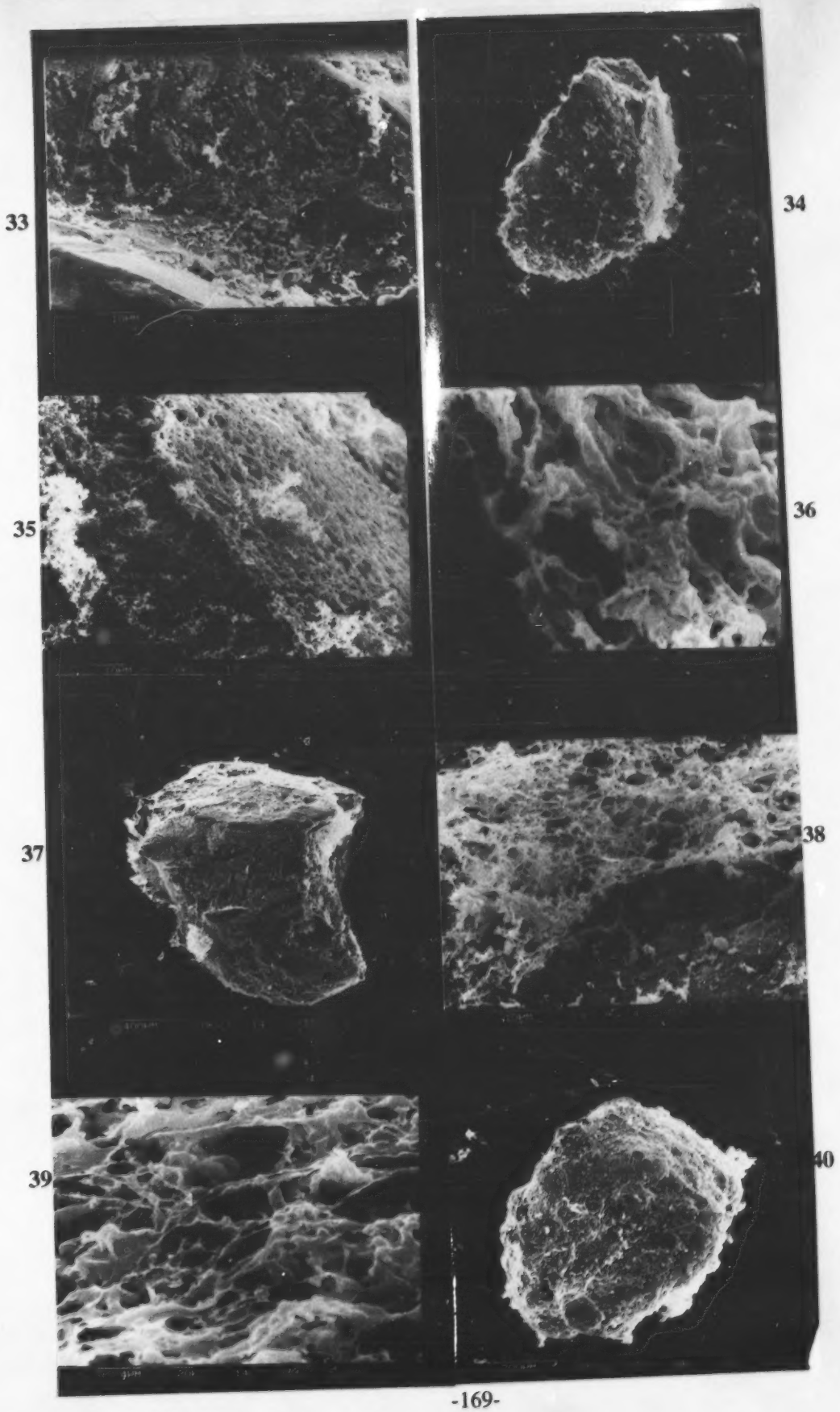
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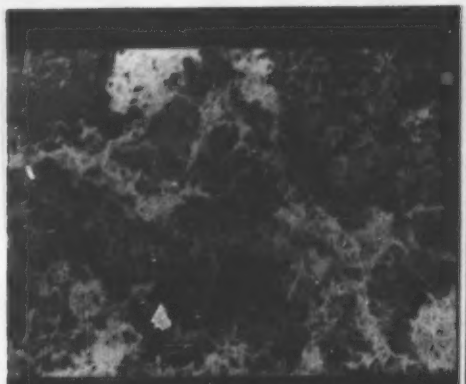

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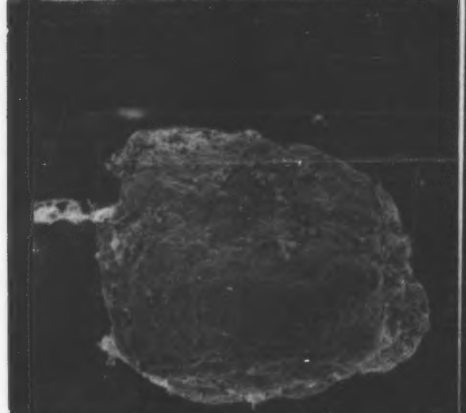

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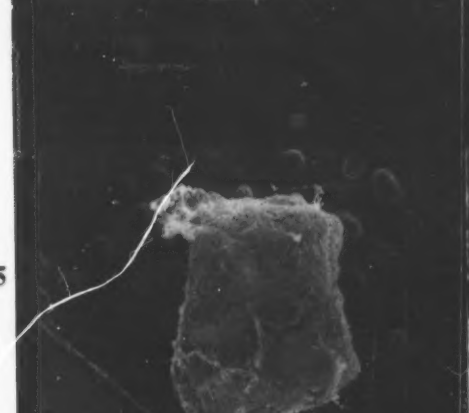

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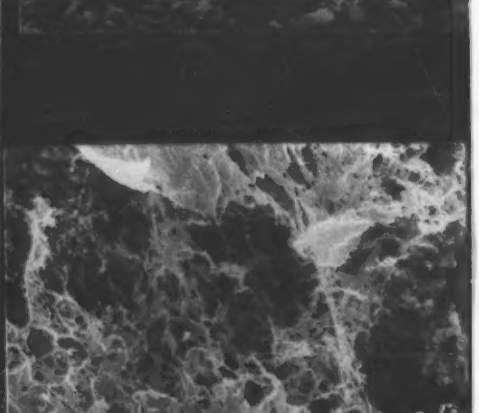

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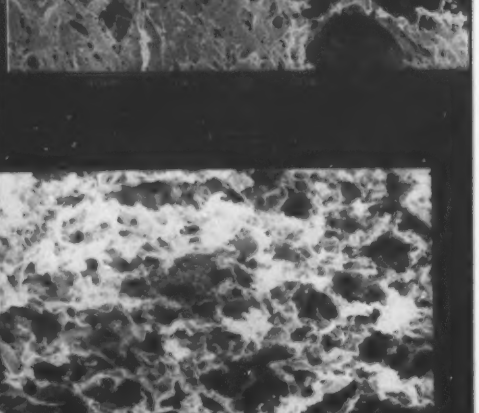

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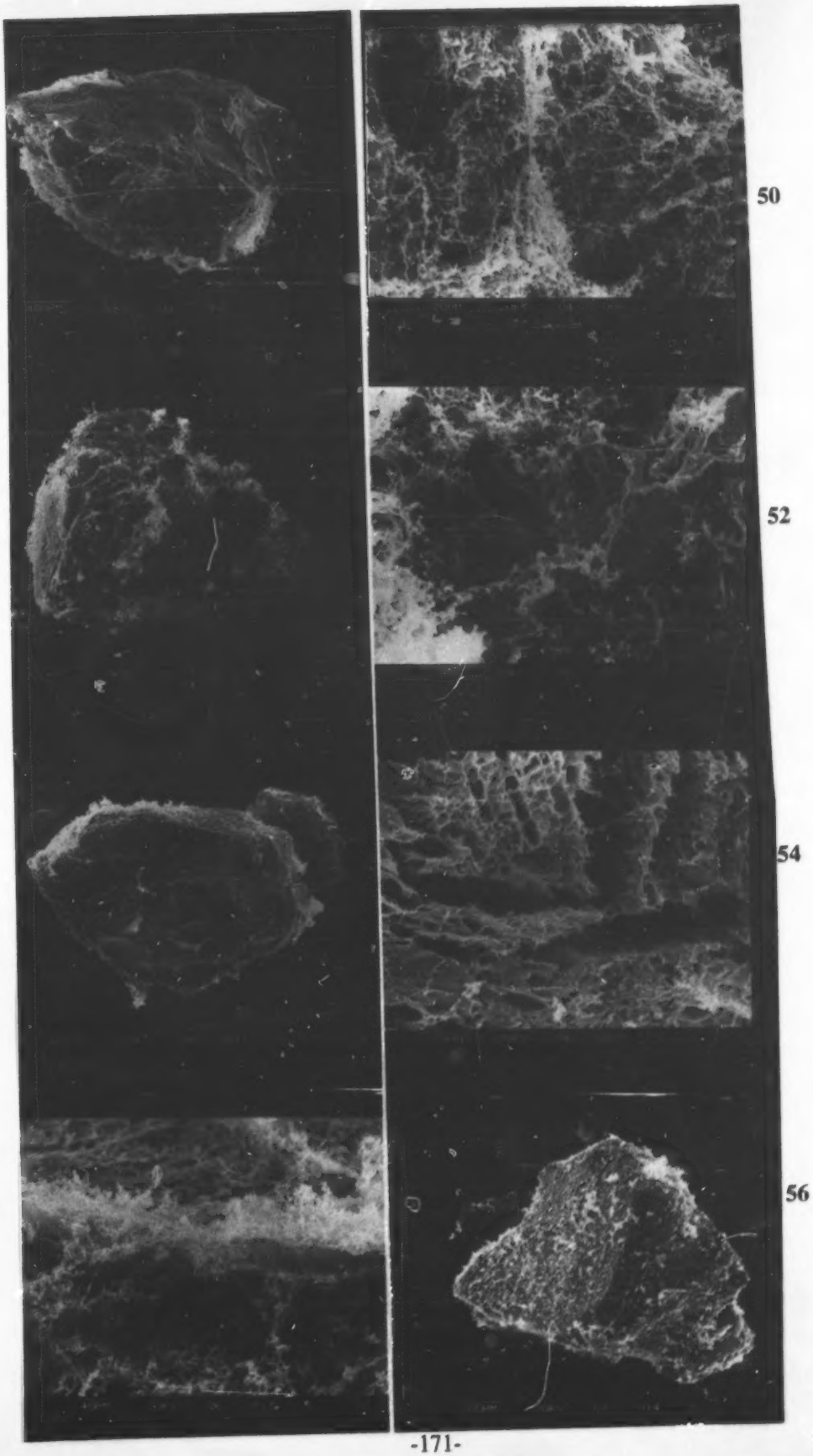

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SEM PHOTOGRAPHS OF SAMPLES ON MARCH 10, 1993

Number of photo	Biofilm
1	F1-0
2	high manification of 1
3	F1-2
4	high manification of 3
5	F2-0
6	high manification of 5
7	F2-2
8	high manification of 7
9	F3-0
10	high manification of 9
11	high manification of 9
12	F4-0
13	high manification of 12
14	F4-2
15	high manification of 14
16	F5-0
17	high manification of 16
18	high manification of 17
19	F5-2
20	high manification of 19
21	C2-0
22	high manification of 21
23	C2-2
24	high manification of 23
25	high manification of 23
26	C3-0
27	high manification of 26
28	C3-2
29	high manification of 28
30	C3-0
31	high manification of 30
32	C4-0
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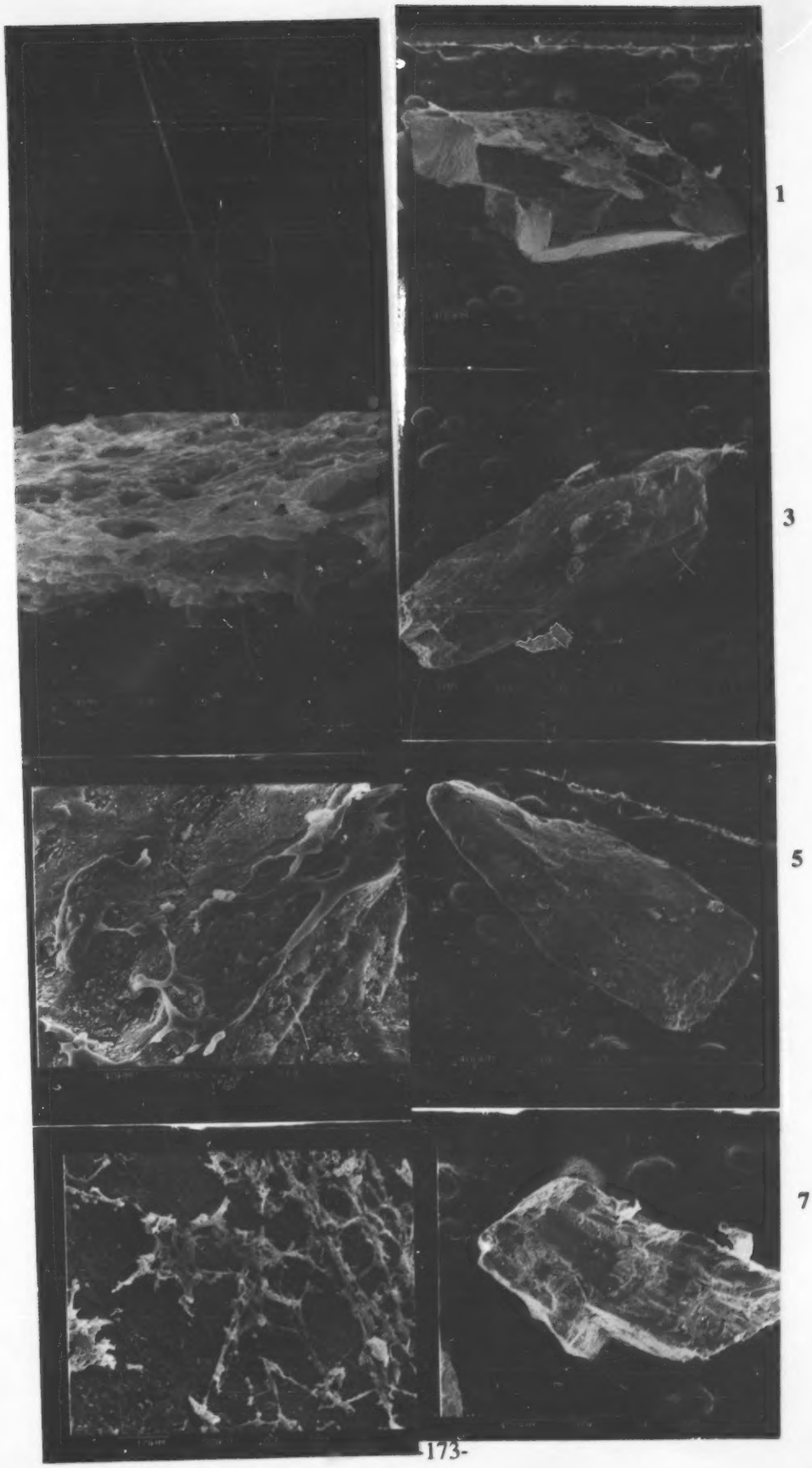
34	C5-0
35	high manification of 34
36	C5-2
37	high manification of 36



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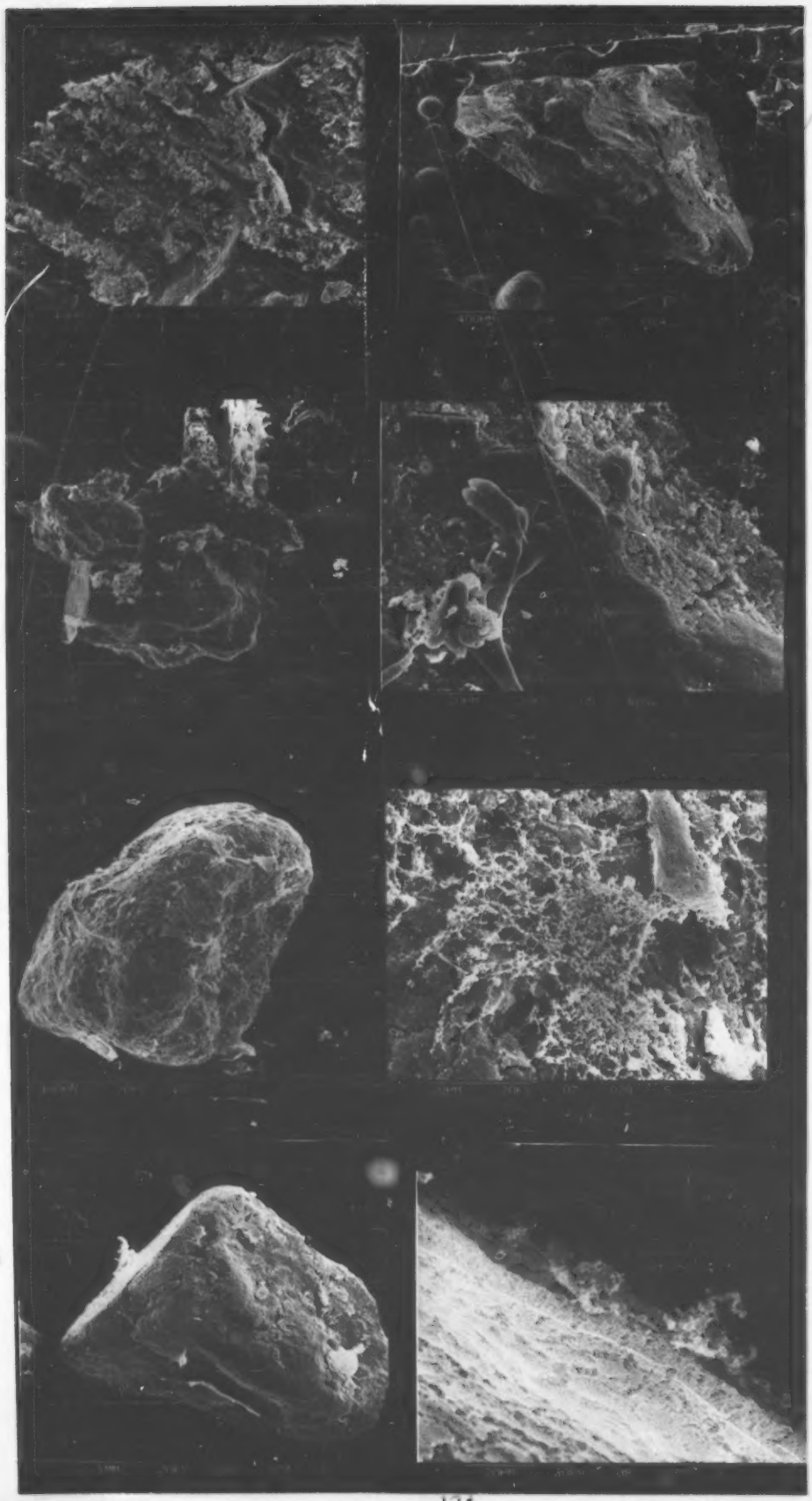

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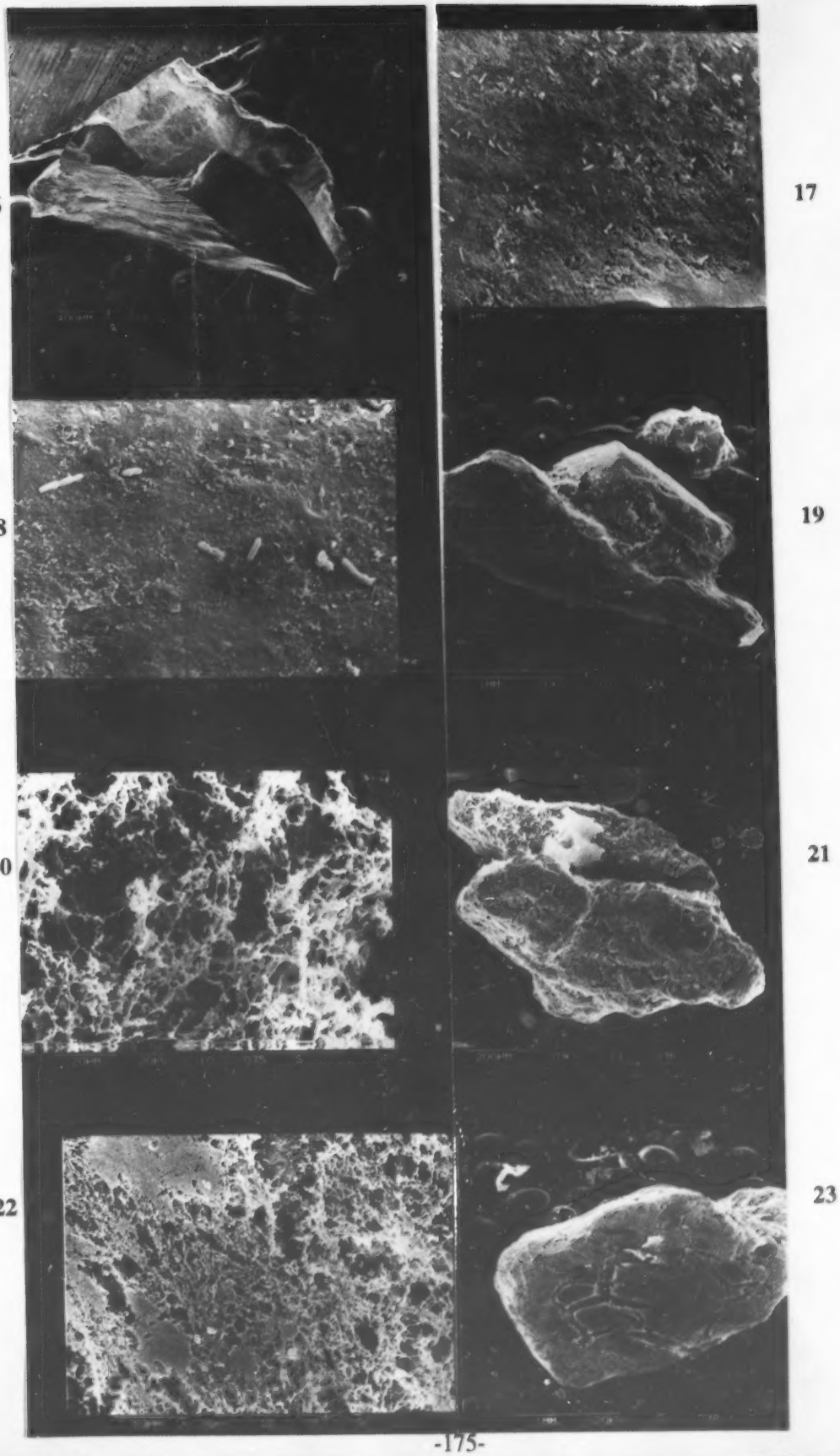

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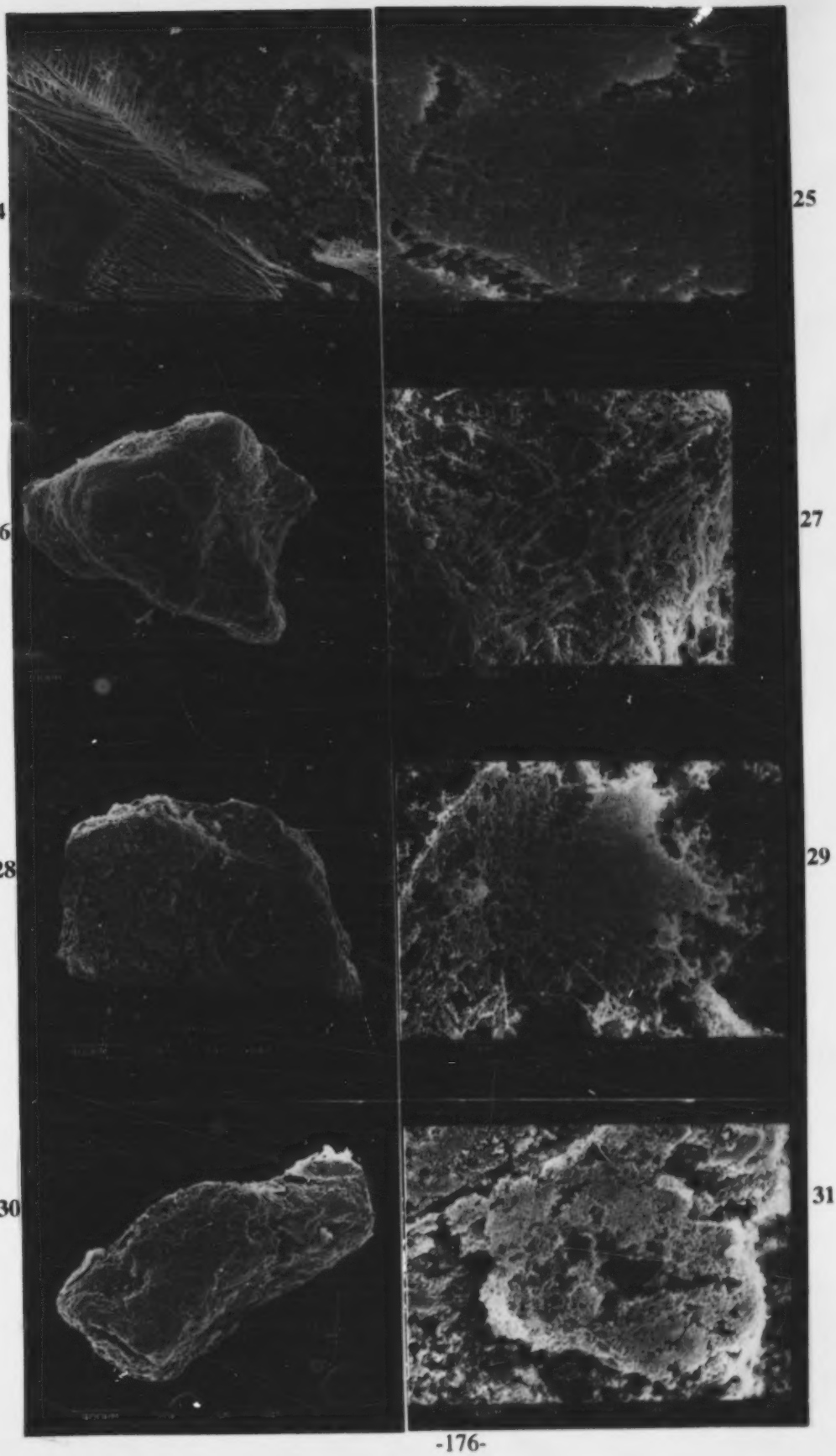

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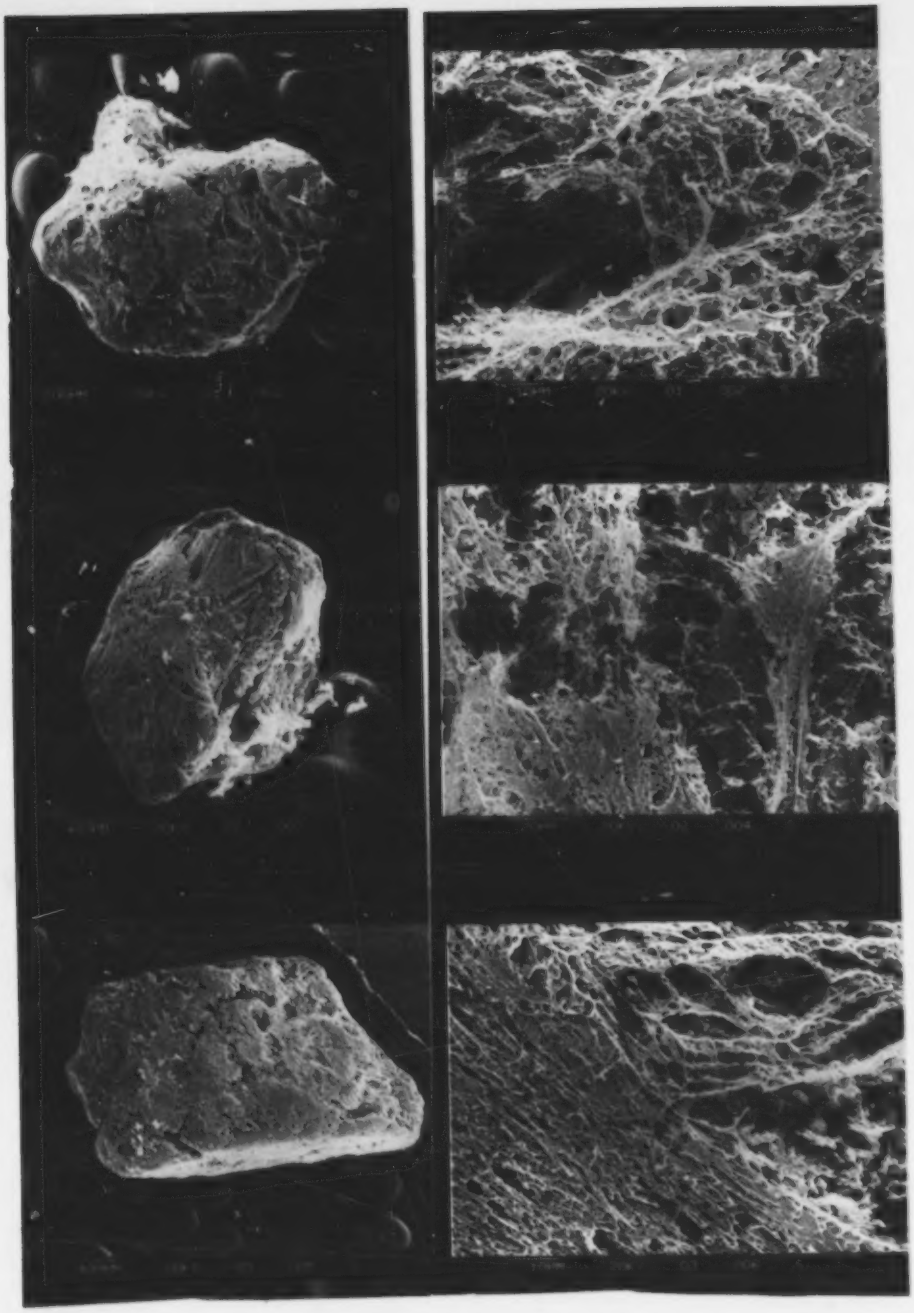

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SEM PHOTOGRAPHS OF SAMPLES ON MARCH 18, 1993

Before Backwashing

Number of photo	Biofilm
1	F1-0
2	high manification of 1
3	F1-2
4	high manification of 3
5	F2-0
6	high manification of 5
7	F2-2
8	high manification of 7
9	F3-0
10	F3-0
11	high manification of 9
12	F3-2
13	high manification of 12
14	F3-2
15	high manification of 14
16	high manification of 15
17	F4-0
18	high manification of 17
19	F4-2
20	high manification of 19
21	F5-0
22	high manification of 21
23	F5-2
24	high manification of 23
25	C2-0
26	high manification of 25
27	C2-2
28	high manification of 27
29	C3-0
30	high manification of 29
31	C3-2
32	high manification of 30
33	C4-0
34	high manification of 33
35	C4-2
36	high manification of 35

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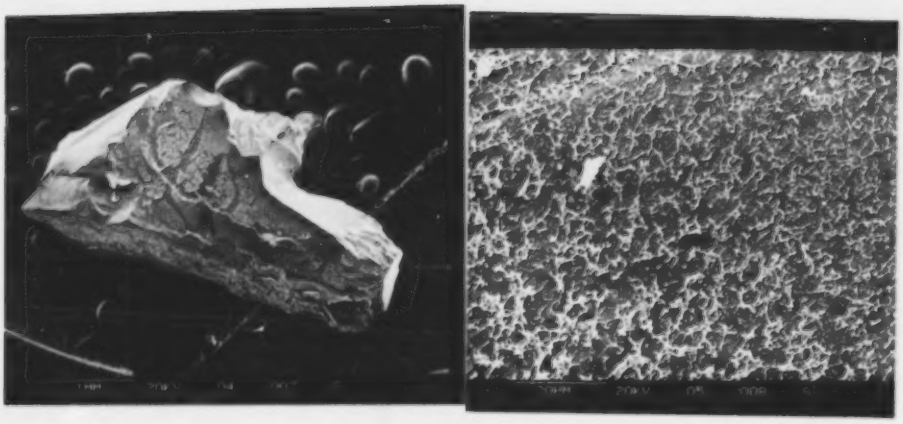

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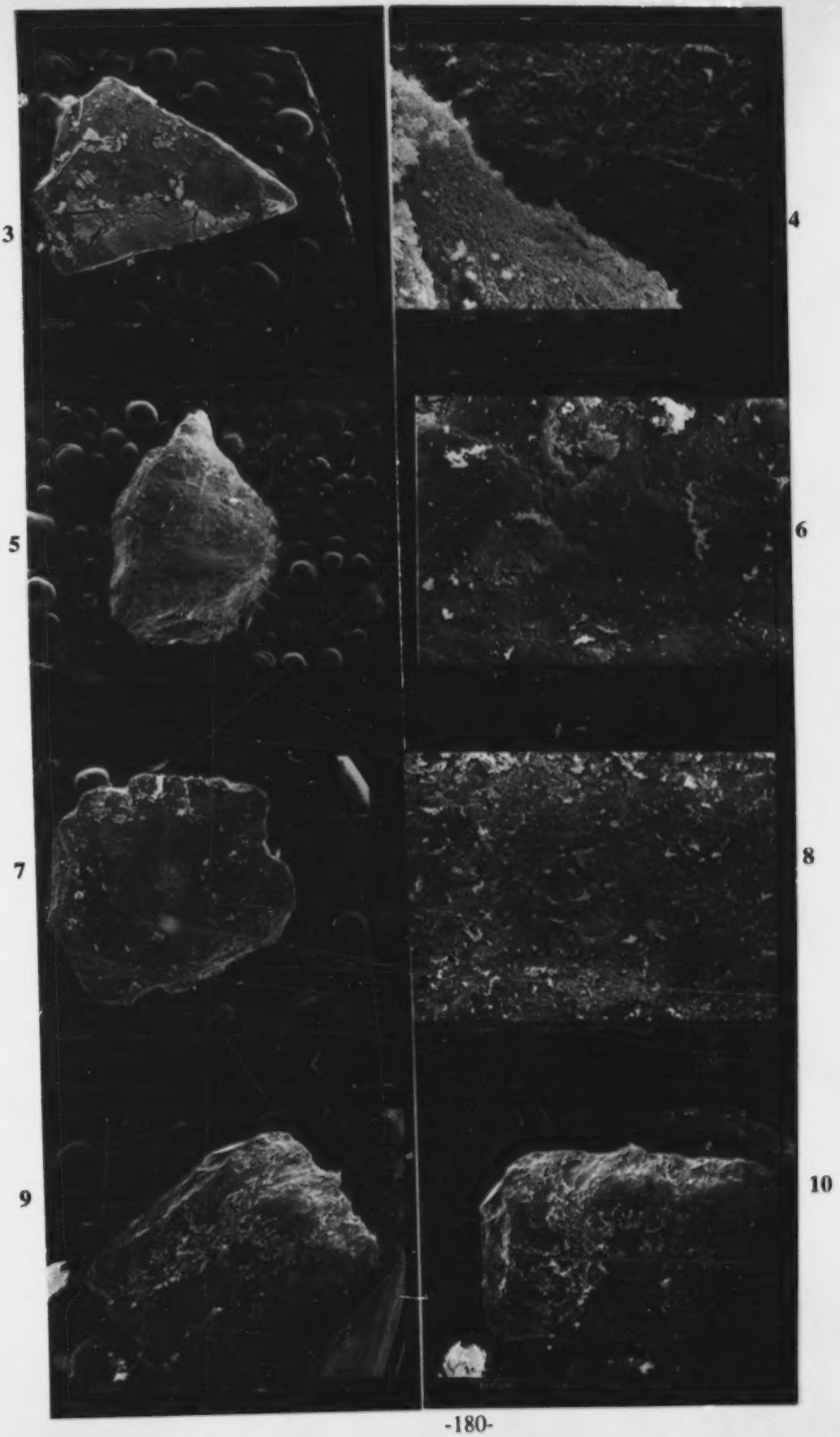
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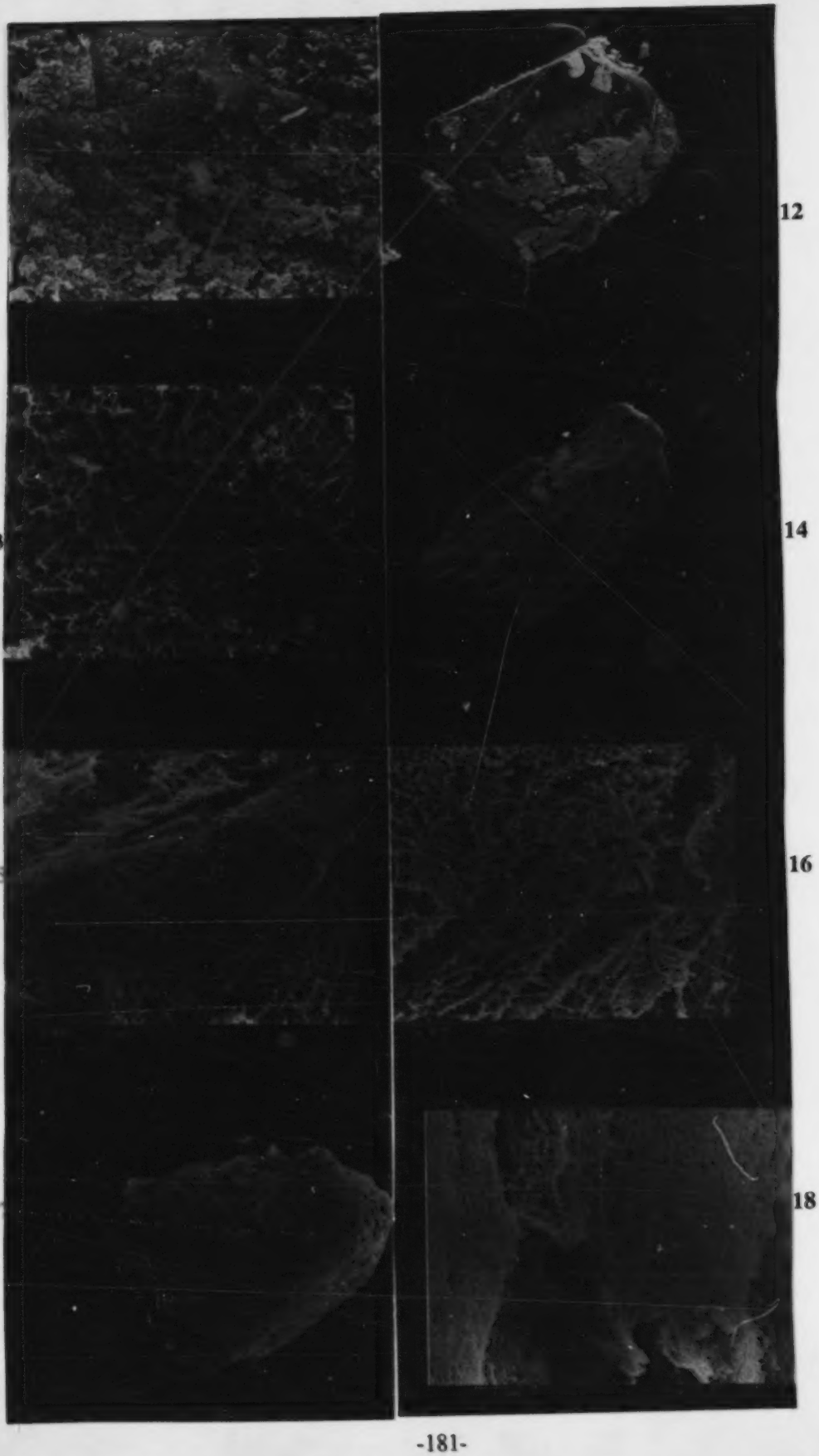
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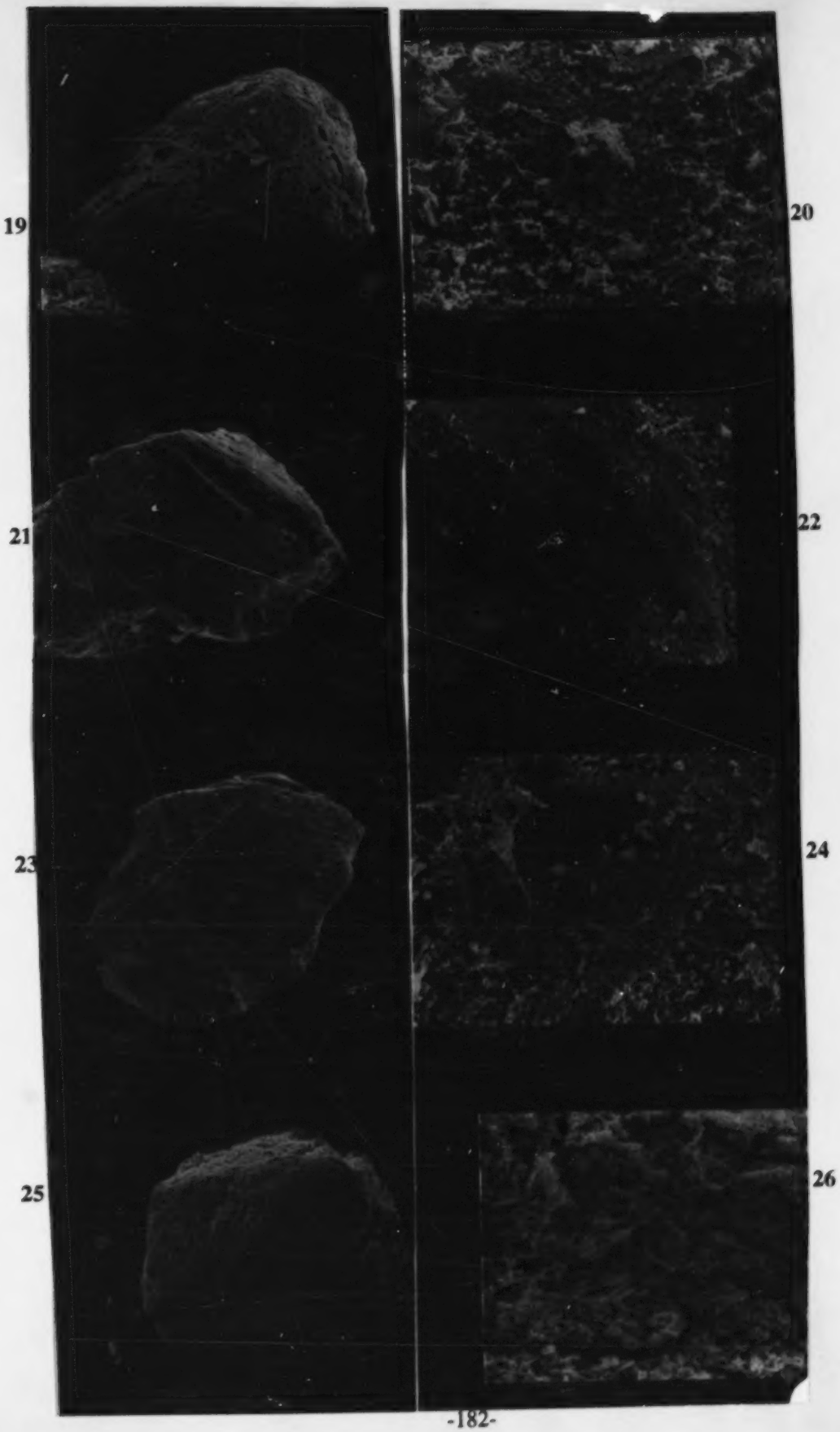
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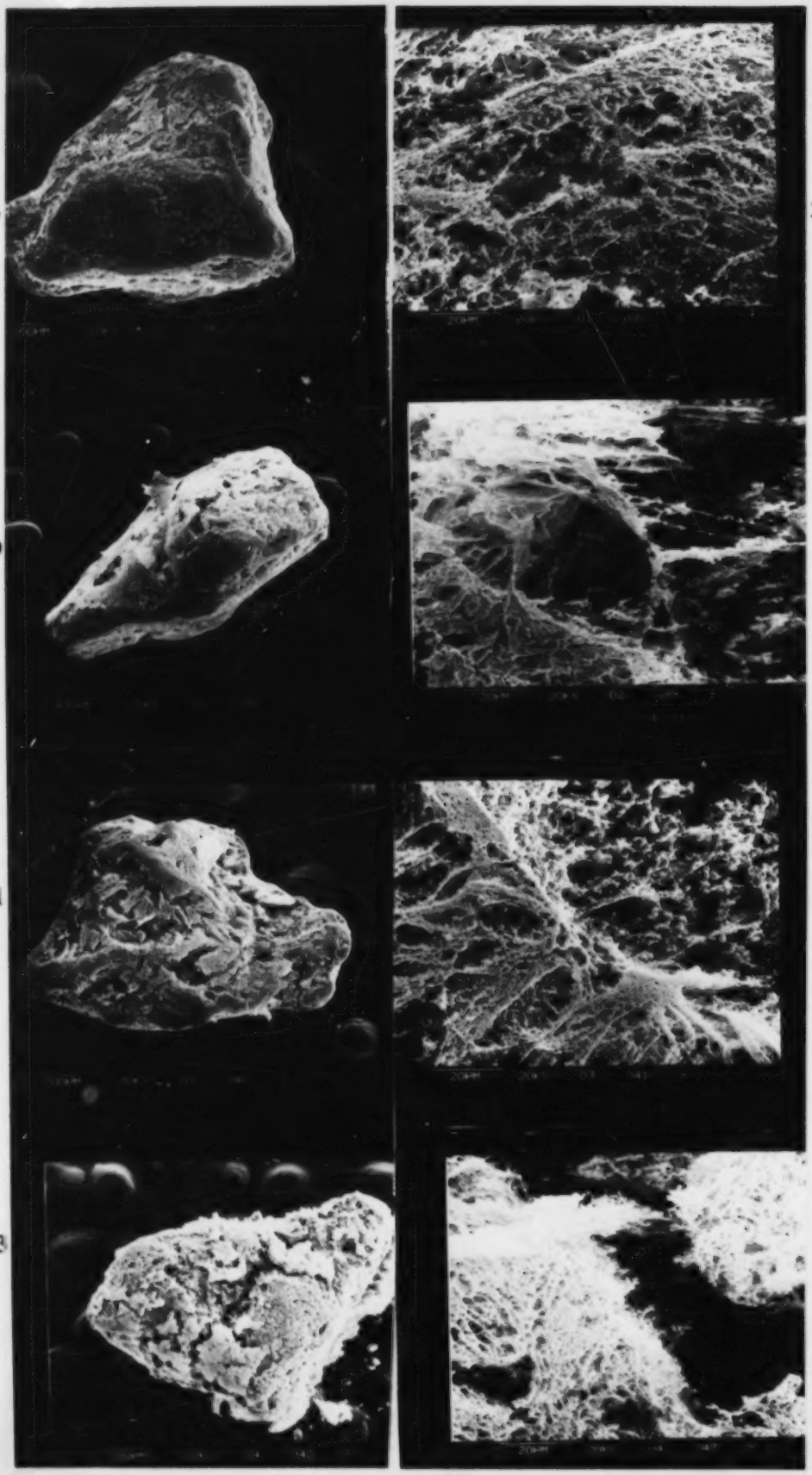

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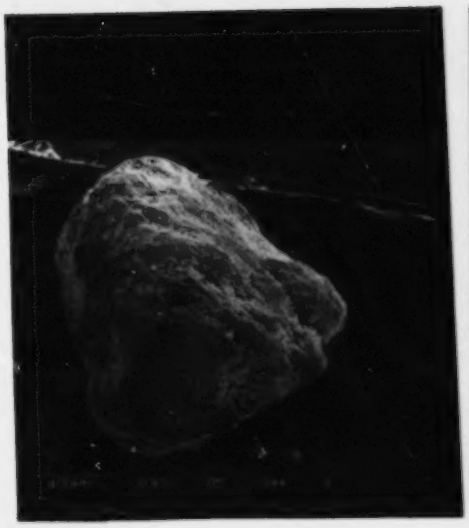

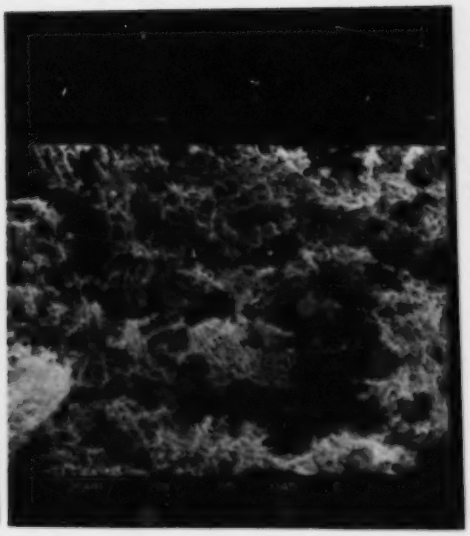

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SEM PHOTOGRAPHS OF SAMPLES ON MARCH 18, 1993

After Backwashing

Number of photo	Biofilm
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2	high manification of 1
3	F1-2
4	high manification of 3
5	F2-0
6	high manification of 5
7	F2-2
8	high manification of 7
9	F3-0
10	high manification of 9
11	F3-2
12	high manification of 11
13	F4-0
14	high manification of 13
15	F4-2
16	high manification of 15
17	F5-0
18	high manification of 17
19	C2-0
20	high manification of 19
21	C3-0
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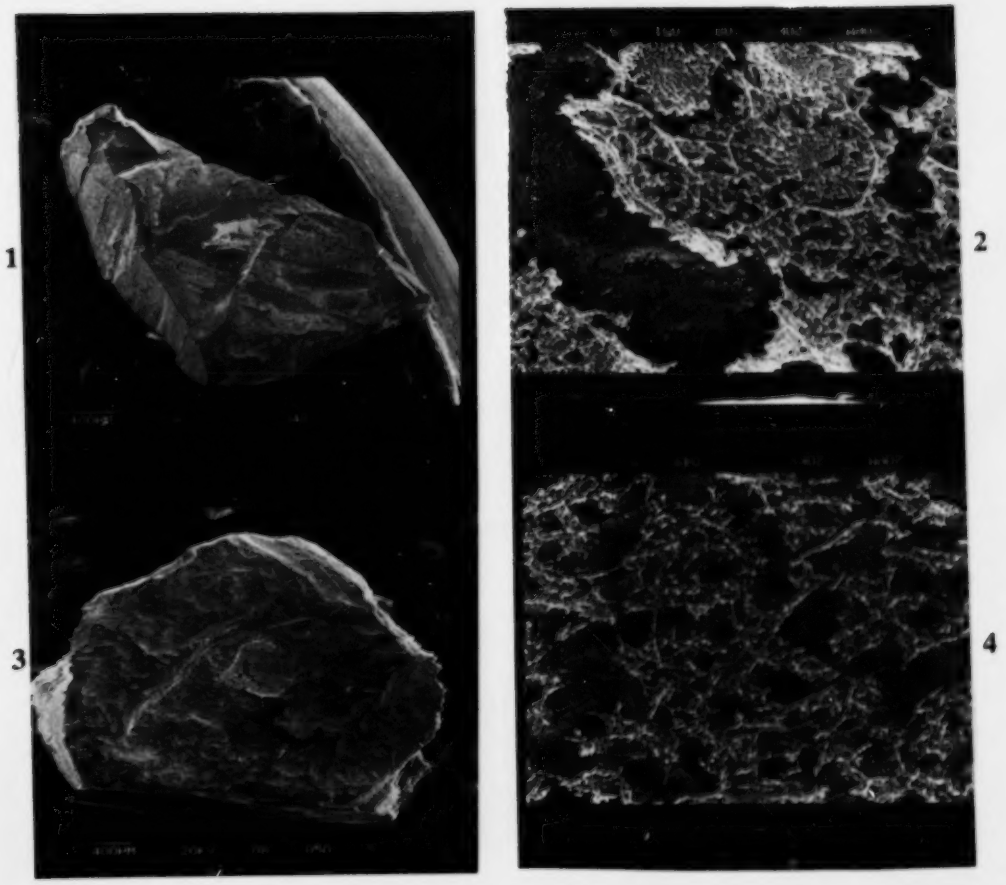
23	C4-0
24	high manification of 23
25	C5-0
26	high manification of 25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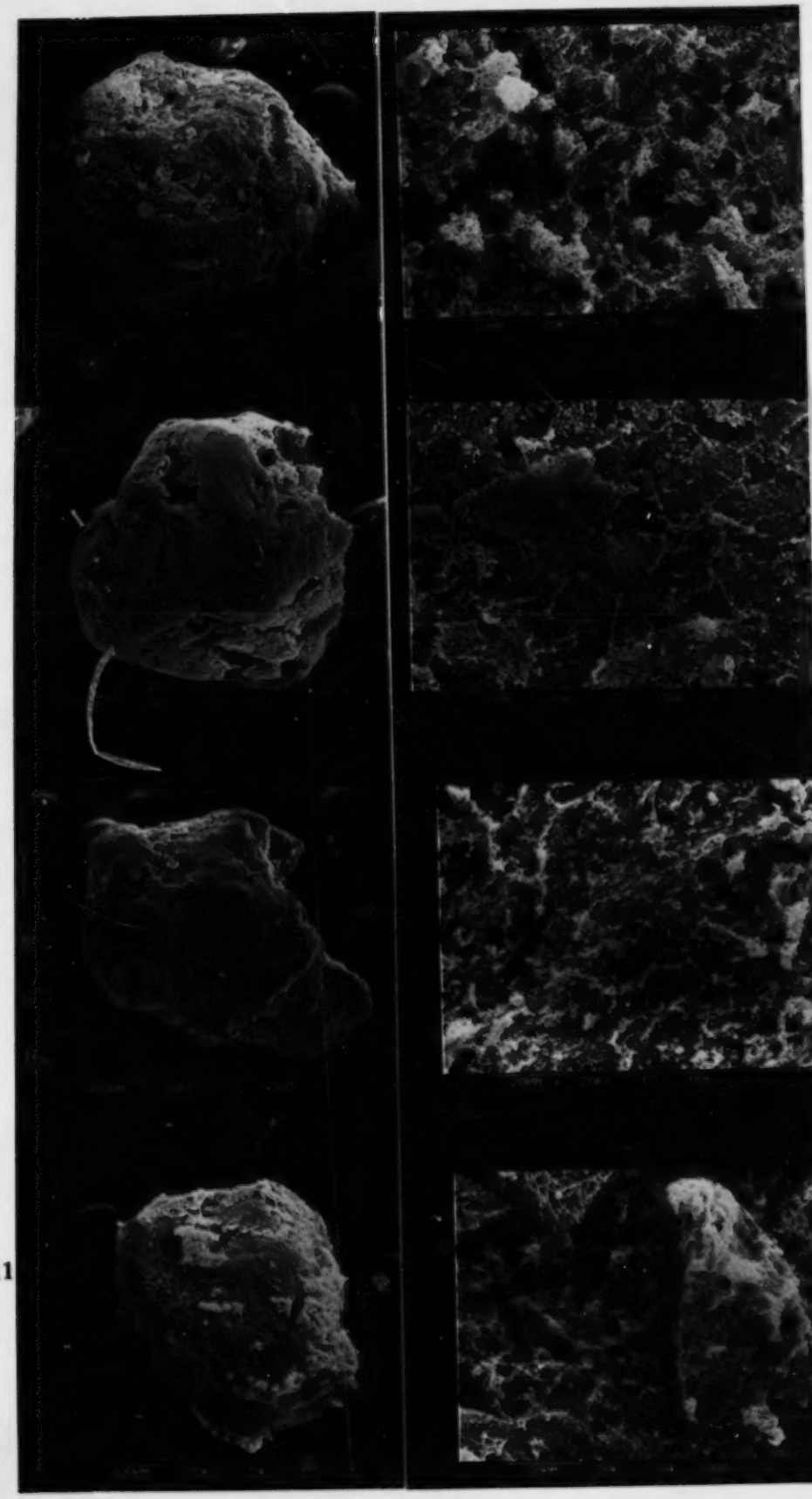

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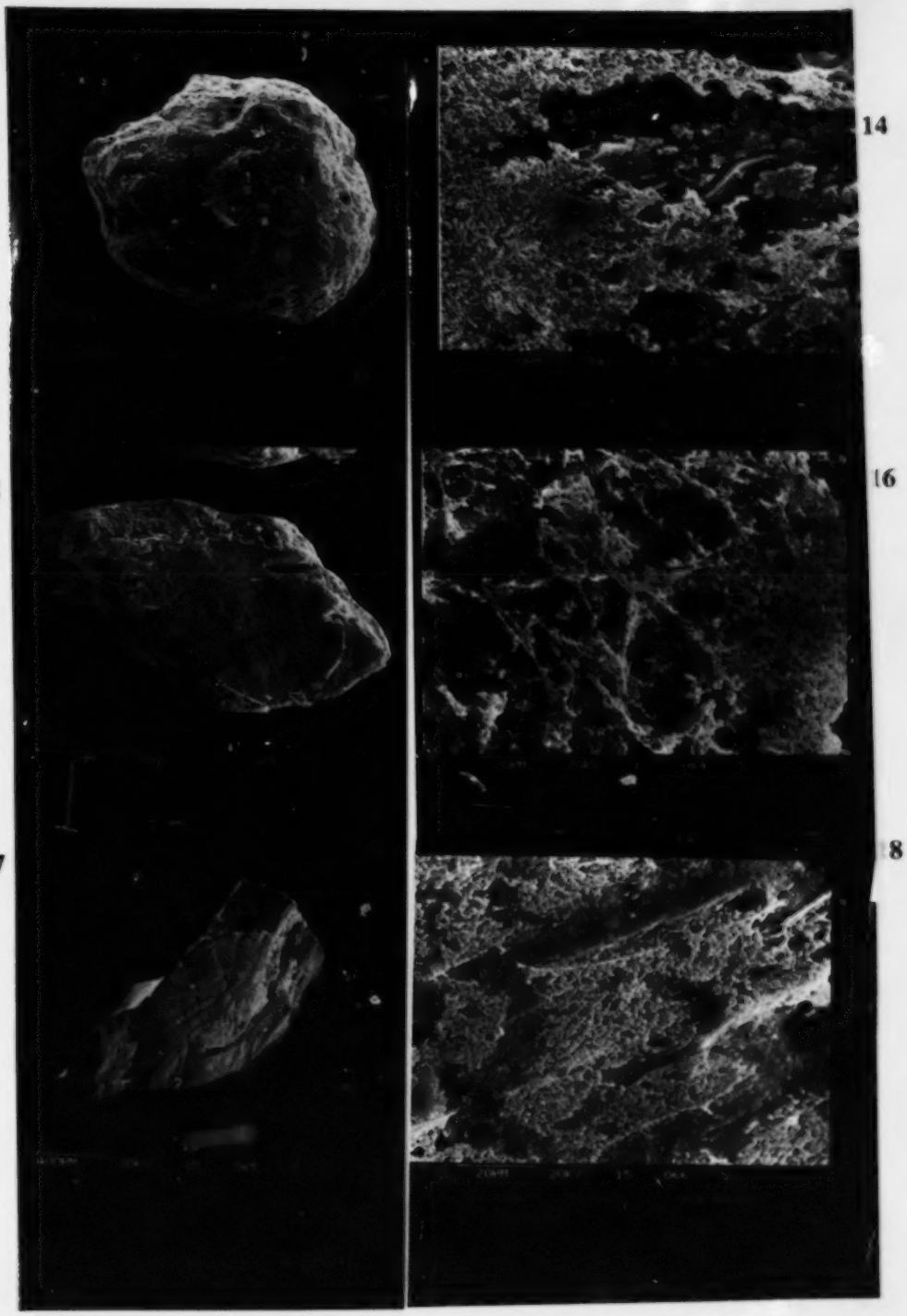
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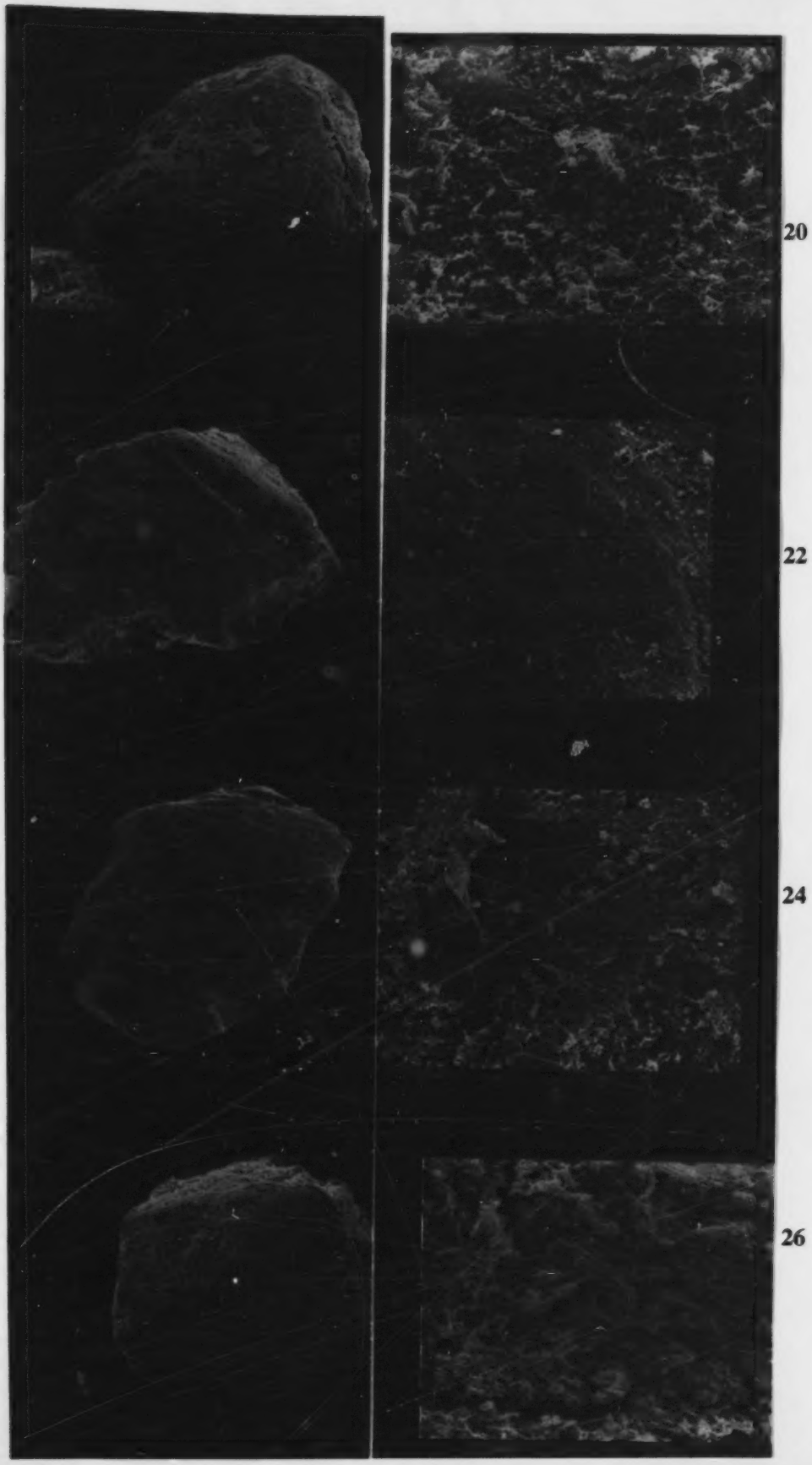

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SEM PHOTOGRAPHS OF SAMPLES ON MARCH 26, 1993

Number of photo	Biofilm
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4	F1-2
5	high manification of 4
6	F2-0
7	high manification of 6
8	F2-2
9	high manification of 8
10	F3-0
11	high manification of 10
12	F4-0
13	high manification of 12
14	F4-2
15	high manification of 14
16	F5-0
17	high manification of 16
18	F5-2
19	high manification of 18
20	C2-0
21	high manification of 20
22	C2-2
23	high manification of 22
24	C3-0
25	high manification of 24
26	C3-2
27	high manification of 26
28	C4-0
29	high manification of 28
30	C4-2
31	high manification of 30
32	C5-0
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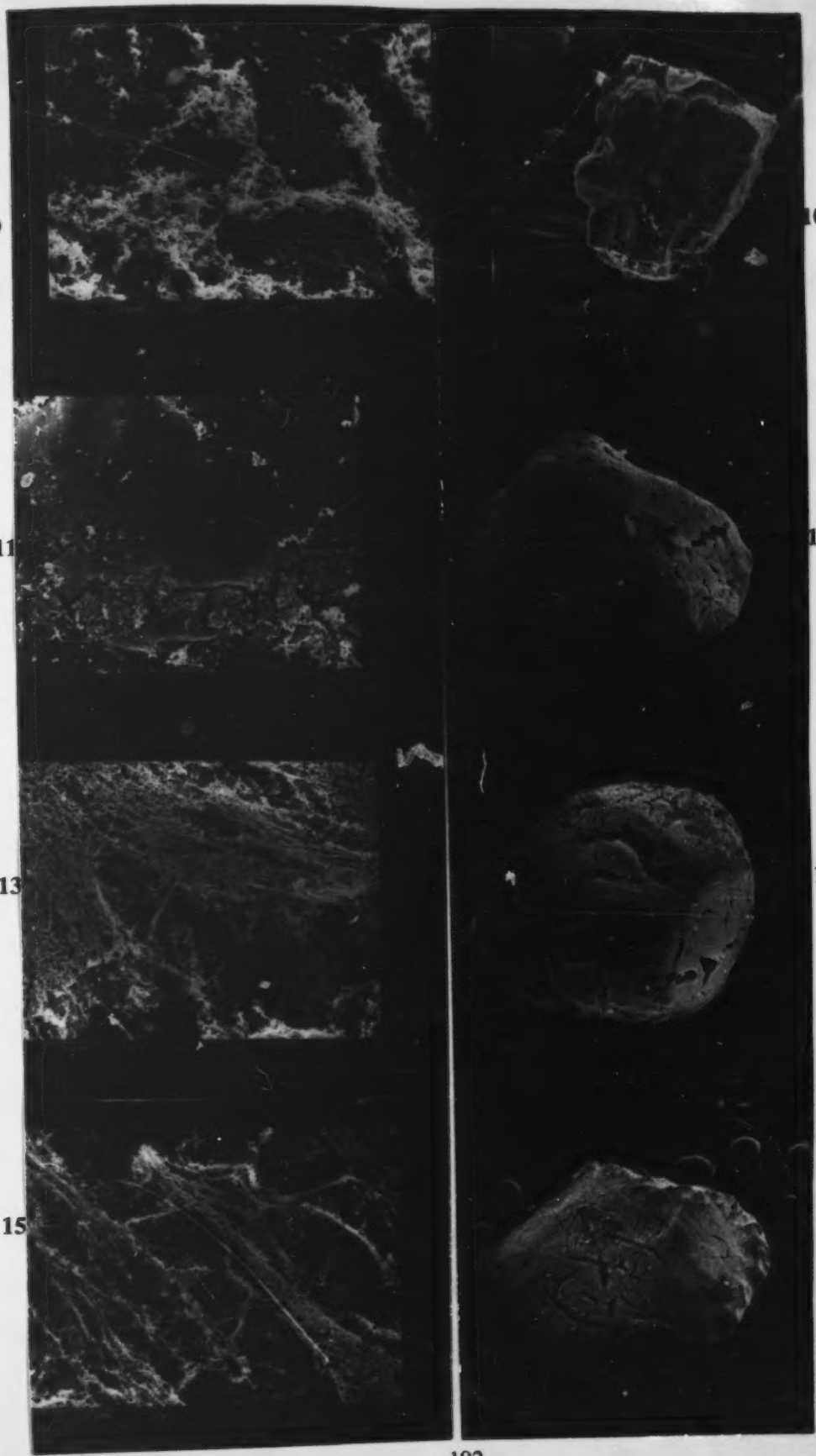
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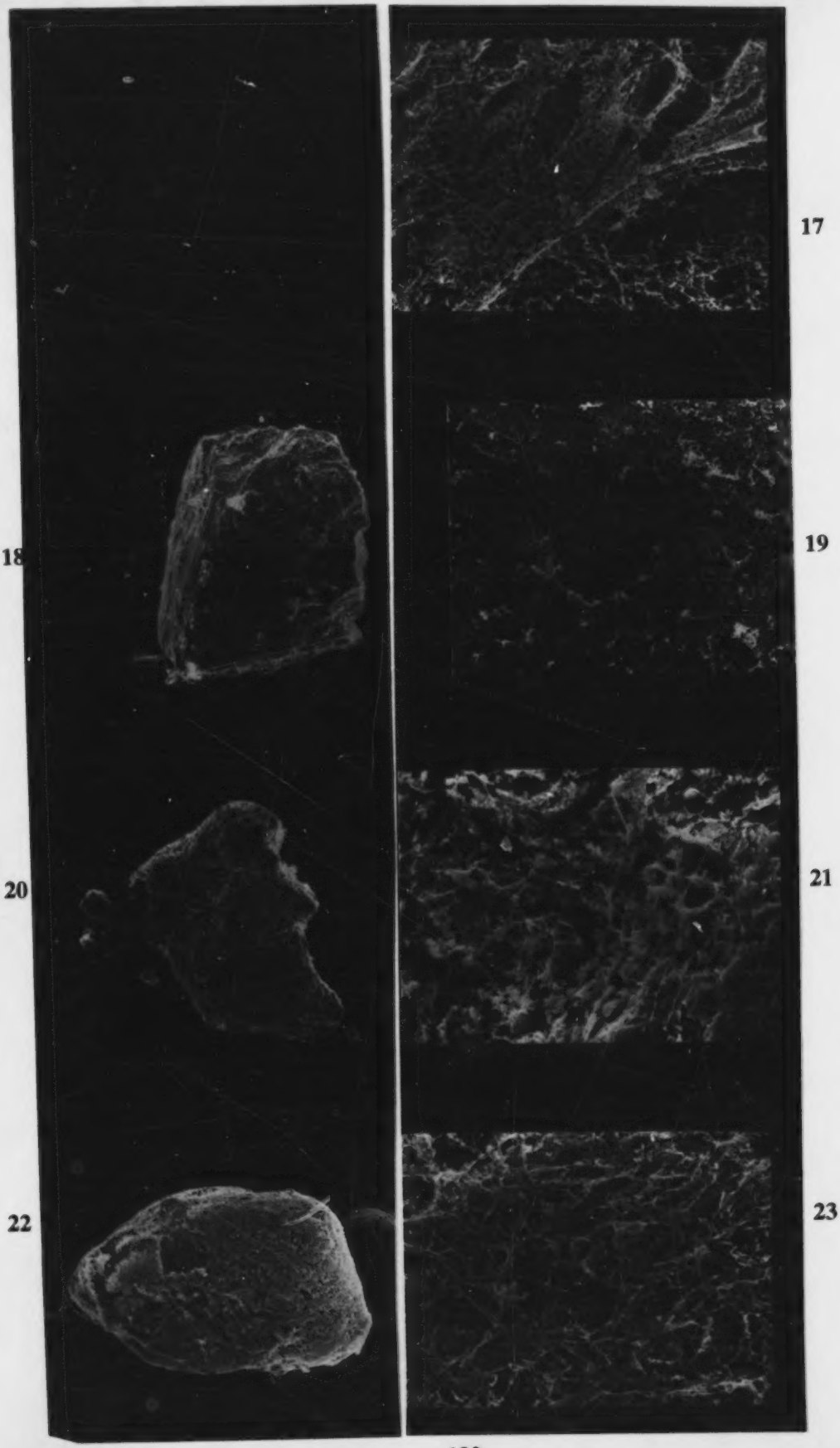

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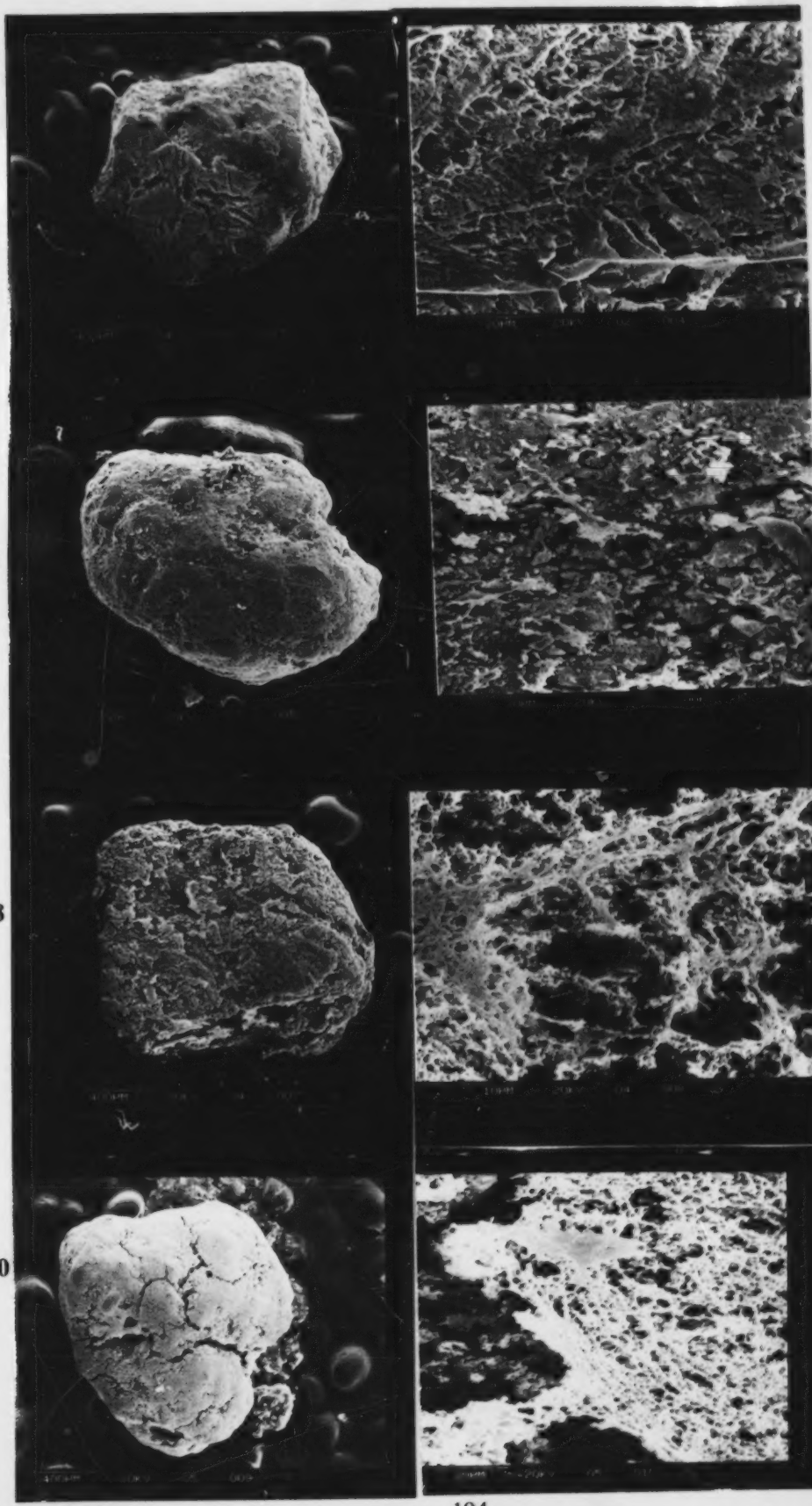

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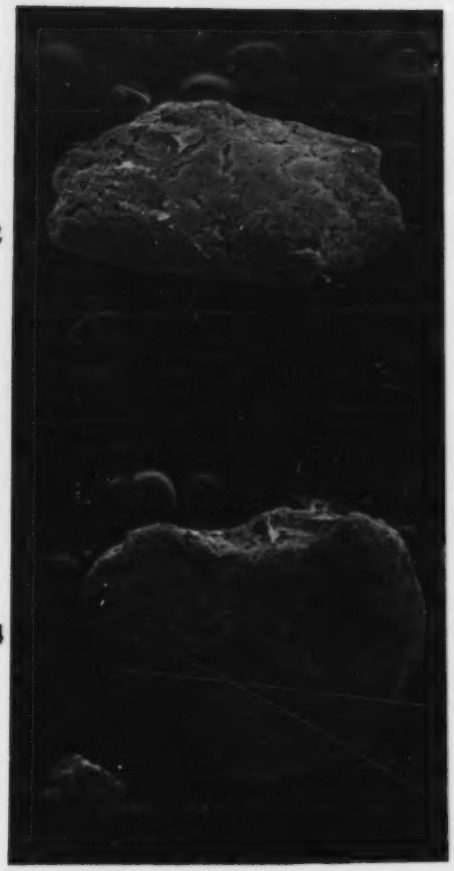

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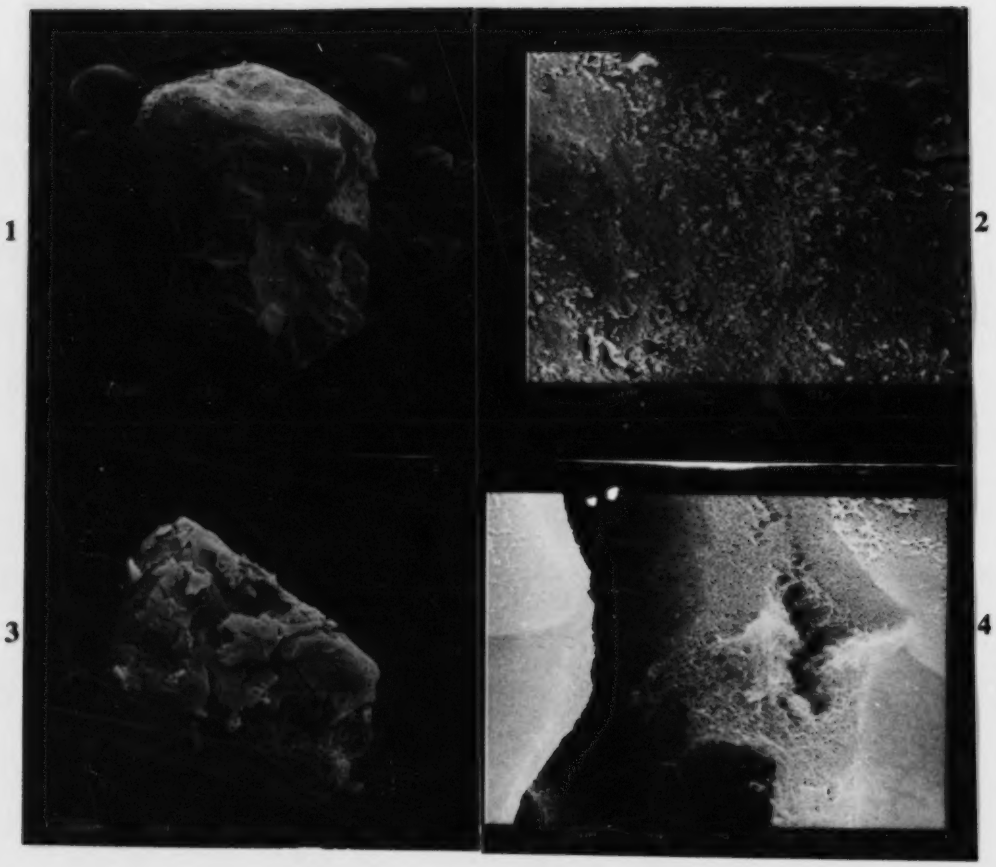
SEM PHOTOGRAPHS OF SAMPLES ON MARCH 29, 1993

Number of photo	Biofilm
1	F2-0
2	high manification of 1
3	F2-2
4	high manification of 3
5	high manification of 3
6	C2-0
7	high manification of 6
8	C2-2
9	high manification of 8



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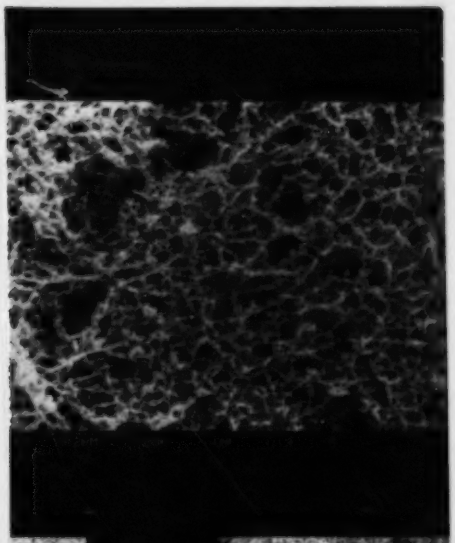

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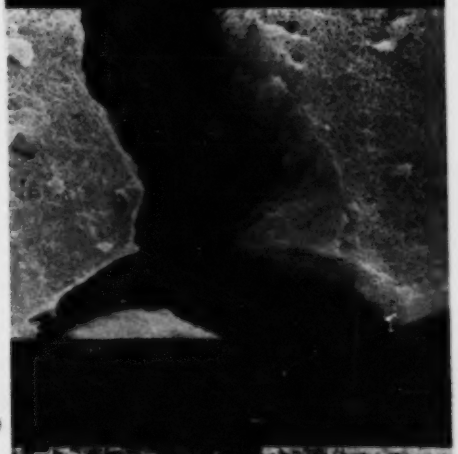

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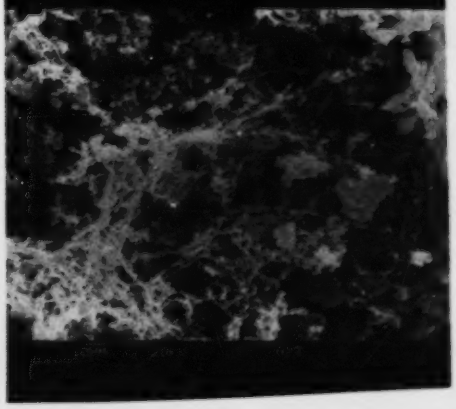
8



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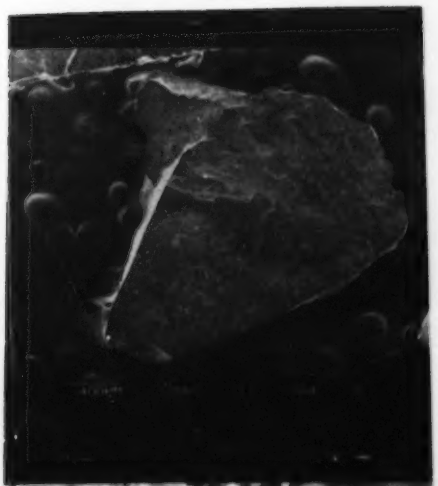
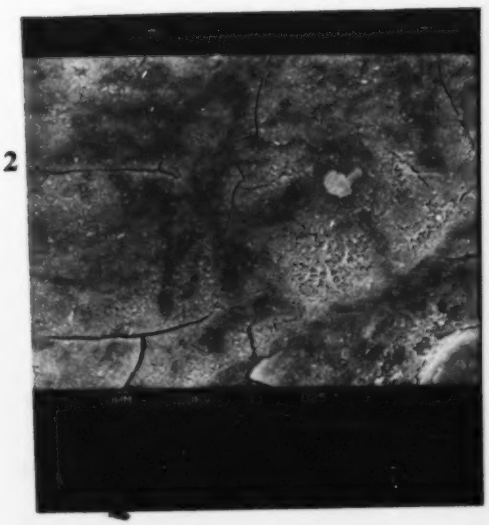
SEM PHOTOGRAPHS OF SAMPLES ON APRIL 1, 1993

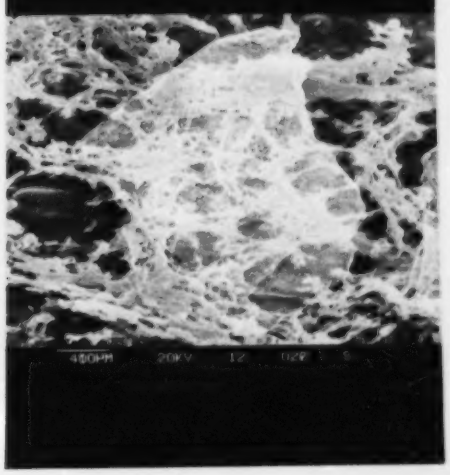
Number of photo	Biofilm
1	F1-0
2	high manification of 1
3	F1-2
4	F2-0
5	high manification of 4
6	F2-2
7	high manification of 6
8	C2-0
9	high manification of 8
10	C2-2
11	high manification of 10



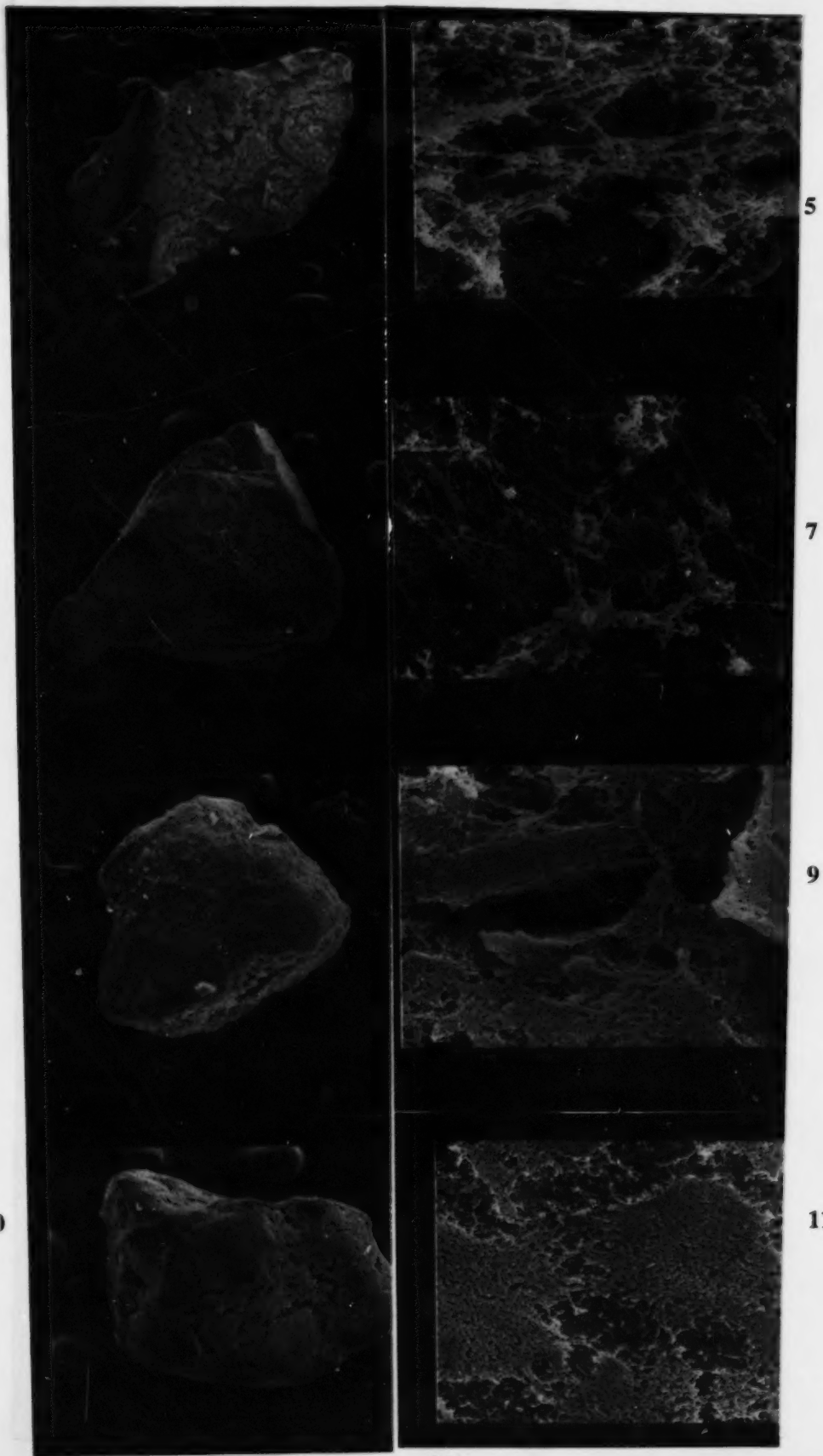
1



2



3



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Appendix B

Biomass Data of Each Sampling Day

			Biomass on Febuary 25, 1993					
Sample*	Weight (g)	Chloroform (ml)	#Absorbance	Conc. (n mole)	TC. (n mole)	pl. n mole/g media	Average	S
F1-0 (1)	1.2261	1	0.005	0.63	2.52	2.06		
							2.61	0.77
F1-0 (2)	0.8005	1	0.005	0.63	2.52	3.15		
F1-2 (1)	0.4997	1	NA	#VALUE!	#VALUE!	#VALUE!		
							#######	#####
F1-2 (2)	0.6531	1	0.007	0.83	3.30	5.05		
F1-3 (1)	0.7307	1	NA	#VALUE!	#VALUE!	#VALUE!		
							#######	#####
F1-3 (2)	0.7119	1	0.001	0.24	0.97	1.36		
F2-0 (1)-	1.0377	1	0.014	1.50	6.02	5.80		
							5.27	0.76
F2-0 (2)	0.7795	1	0.008	0.92	3.69	4.73		
F2-2 (1)-	0.5841	1	0.004	0.53	2.14	3.66		
							4.02	0.51
F2-2 (2)	0.6645	1	0.006	0.73	2.91	4.38		
F2-3 (1)	0.8771	1	0.008	0.92	3.69	4.21		
							4.64	0.61
F2-3 (2)	0.7271	1	0.008	0.92	3.69	5.07		
F3-0 (1)	1.0080	1	0.008	0.92	3.69	3.66		

							########	#####
F3-0 (2)-	0.8536	1	NA	#VALUE!	#VALUE!	#VALUE!		
F3-2 (1)	0.5121	1	0.002	0.34	1.36	2.65		
F3-2 (2)	0.6097	1	NA	NA	#VALUE!	#VALUE!	########	#####
F3-3 (1)	0.5496	1	0.002	0.34	1.36	2.47		
F3-3 (2)	0.5391	1	0.002	0.34	1.36	2.52	2.50	0.03
F4-0 (1)	0.5908	1	NA	#VALUE!	#VALUE!	#VALUE!		
F4-0 (2)-	0.7051	1	0.006	0.73	2.91	4.13	########	#####
F4-2 (1)	0.3694	1	0.001	0.24	0.97	2.63		
F4-3 (1)	0.6731	1	0.001	0.24	0.97	1.44		
F4-3 (2)-	0.4925	1	0.001	0.24	0.97	1.97	1.71	0.37
F5-0 (1)-	0.9792	1	0.009	1.02	4.08	4.16		
F5-0 (2)	1.2192	1	0.015	1.60	6.41	5.26	4.71	0.77
F5-2 (1)	0.8792	1	0.008	0.92	3.69	4.20		
F5-2 (2)	0.5142	1	0.004	0.53	2.14	4.15	4.18	0.03

F5-3 (1) -	0.5974	1	0.001	0.24	0.97	1.63		
							2.02	0.55
F5-3 (2)	0.8864	1	0.004	0.53	2.14	2.41		
C2-0 (1)	1.4846	1	NA	#VALUE!	#VALUE!	#VALUE!		
							#######	#####
C2-0 (2)	1.2859	1	0.011	1.21	4.85	3.78		
C2-2 (1)	1.2439	1	NA	#VALUE!	#VALUE!	#VALUE!		
							#######	#####
C2-2 (2)	1.1645	1	0.007	0.83	3.30	2.83		
C3-0 (1)	1.5590	1	0.020	2.09	8.35	5.36		
							#######	#####
C3-0 (2)	0.9318	1	NA	#VALUE!	#VALUE!	#VALUE!		
C3-1 (1)	0.9594	1	0.010	1.12	4.47	4.66		
							4.56	0.13
C3-1 (2)	0.5651	1	0.005	0.63	2.52	4.47		
C3-2 (1)	1.0604	1	0.006	0.73	2.91	2.75		
							2.86	0.16
C3-2 (2)	1.2384	1	0.008	0.92	3.69	2.98		
C4-0 (1)-	0.9400	1	0.015	1.60	6.41	6.82		
							7.03	0.30
C4-0 (2)	0.8310	1	0.014	1.50	6.02	7.24		

C4-2 (1)	1.2883	1	0.001	0.24	0.97	0.75		
							0.76	0.01
C4-2 (2)-	1.7670	1	0.002	0.34	1.36	0.77		
C5-0 (1)	0.5244	1	0.007	0.83	3.30	6.29		
							6.21	0.12
C5-0 (2)	0.5388	1	0.007	0.83	3.30	6.13		
C5-1 (1)	0.6305	1	0.003	0.44	1.75	2.77		
							2.78	0.01
C5-1 (2)	0.7654	1	0.004	0.53	2.14	2.79		
C5-2 (1)	0.9101	1	0.007	0.83	3.30	3.63		
							#######	#####
C5-2 (2)	1.1415	1	NA	#VALUE!	#VALUE!	#VALUE!		
blank		0						
Total chroform=4 ml.								

			Biomass on March 3, 1993					
Sample*	Weight (g)	Chloroform (ml)	#Absorbance	Conc. (n mole)	TC. (n mole)	pl. n mole/g media	Average	S
F2-0 (1)-	0.8438	1	0.001	0.24	0.97	1.15		
							1.31	0.23
F2-0 (2)	0.9209	1	0.002	0.34	1.36	1.48		
F2-2 (2)	1.0309	1	0.006	0.73	2.91	2.83		
F2-3 (1)	1.7774	1	0.010	1.12	4.47	2.51		
							#######	#####
F2-3 (2)	1.7029	1	NA	#VALUE!	#VALUE!	#VALUE!		
F3-0 (1)	1.0548	1	NA	#VALUE!	#VALUE!	#VALUE!		
							#######	#####
F3-0 (2)-	1.0219	1	0.010	1.12	4.47	4.37		
F3-2 (1)	0.9054	1	0.006	0.73	2.91	3.22		
							3.58	0.52
F3-2 (2)	0.9337	1	0.008	0.92	3.69	3.95		
F3-3 (1)	0.9500	1	NA	#VALUE!	#VALUE!	#VALUE!		
							#######	#####
F3-3 (2)	0.9673	1	0.007	0.83	3.30	3.41		
F4-0 (1)	0.6560	1	0.010	1.12	4.47	6.81		
							6.92	0.16
F4-0 (2)-	0.6896	1	0.011	1.21	4.85	7.04		

F4-2 (1)	0.9678	1	0.007	0.83	3.30	3.41		
							2.24	1.65
F4-2 (2)	0.9033	1	0.001	0.24	0.97	1.07		
F4-3 (1)	1.0359	1	0.003	0.44	1.75	1.69		
							1.74	0.08
F4-3 (2)-	1.1882	1	0.004	0.53	2.14	1.80		
F5-0 (1)-	0.6696	1	0.003	0.44	1.75	2.61		
							2.73	0.17
F5-0 (2)	0.7493	1	0.004	0.53	2.14	2.85		
F5-2 (1)	0.8617	1	0.000	0.15	0.58	0.68		
							0.80	0.17
F5-2 (2)	1.0618	1	0.001	0.24	0.97	0.91		
F5-3 (1)	1.1076	1	0.000	0.15	0.58	0.53		
							0.59	0.09
F5-3 (2)	0.8943	1	0.000	0.15	0.58	0.65		
C2-0 (1)	1.1339	1	0.025	2.57	10.29	9.08		
							8.76	0.45
C2-0 (2)	0.6676	1	0.013	1.41	5.63	8.43		
C2-2 (1)	1.6647	1	NA	#VALUE!	#VALUE!	#VALUE!		
							#######	######
C2-2 (2)	1.8743	1	0.009	1.02	4.08	2.18		

C3-0 (1)	1.1040	1	0.017	1.80	7.18	6.51		
							6.18	0.47
C3-0 (2)	1.3623	1	0.019	1.99	7.96	5.84		
C3-1 (1)	0.8225	1	NA	#VALUE!	#VALUE!	#VALUE!		
							#######	#####
C3-1 (2)	0.5935	1	0.007	0.83	3.30	5.56		
C3-2 (1)	1.4398	1	0.002	0.34	1.36	0.94		
							0.94	0.01
C3-2 (2)	1.4662	1	0.002	0.34	1.36	0.93		
C4-0 (1)-	1.1310	1	0.010	1.12	4.47	3.95		
							#######	#####
C4-0 (2)	1.6508	1	NA	#VALUE!	#VALUE!	#VALUE!		
C4-2 (1)	1.5831	1	0.011	1.21	4.85	3.07		
							3.29	0.32
C4-2 (2)-	1.2691	1	0.010	1.12	4.47	3.52		
C5-0 (1)	1.3473	1	NA	#VALUE!	#VALUE!	#VALUE!		
							#######	#####
C5-0 (2)	0.9909	1	0.000	0.15	0.58	0.59		
C5-1 (1)	0.7551	1	NA	#VALUE!	#VALUE!	#VALUE!		
							#######	#####
C5-1 (2)	0.8379	1	0.007	0.83	3.30	3.94		

C5-2 (1)	1.2551	1	0.005	0.63	2.52	2.01		
							1.42	0.84
C5-2 (2)	1.1854	1	0.001	0.24	0.97	0.82		
blank		0						
Total chroform=4 ml.								

			Biomass on March 10, 1993					
Sample*	Weight (g)	Chloroform (ml)	Absorbance	Conc. (n mole)	TC. (n mole)	pl. n mole/g media	Average	S
F1-0 (1)	0.8771	1	0.009	1.02	4.08	4.65	4.66	0.01
F1-0 (2)	1.1234	1	0.012	1.31	5.24	4.67		
F1-2 (1)	0.7663	1	0.009	1.02	4.08	5.32	5.88	0.79
F1-2 (2)	0.4526	1	0.006	0.73	2.91	6.44		
F1-3 (1)	1.0022	1	0.023	2.38	9.51	9.49	9.93	0.61
F1-3 (2)	0.8433	1	0.021	2.18	8.74	10.36		
F2-0 (1)	0.8803	1	0.011	1.21	4.85	5.51	5.39	0.18
F2-0 (2)	0.8483	1	0.010	1.12	4.47	5.26		
F2-2 (1)	0.4562	1	0.006	0.73	2.91	6.38		
F2-3 (1)	0.7916	1	0.007	0.83	3.30	4.17	4.57	0.56
F2-3 (2)	0.8207	1	0.009	1.02	4.08	4.97		
F3-0 (1)	0.9342	1	0.009	1.02	4.08	4.36	5.44	1.53
F3-0 (2)-	1.2799	1	0.020	2.09	8.35	6.52		

F3-2 (1)	0.9018	1	0.008	0.92	3.69	4.09	3.88	0.30
F3-2 (2)	1.2180	1	0.010	1.12	4.47	3.67		
F3-3 (1)	0.9357	1	0.002	0.34	1.36	1.45	#######	#####
F3-3 (2)	0.7586	1	NA	#VALUE!	#VALUE!	#VALUE!		
F4-0 (1)	0.9176	1	0.010	1.12	4.47	4.87	6.62	2.48
F4-0 (2)-	0.9514	1	0.019	1.99	7.96	8.37		
F4-2 (1)	0.8520	1	0.010	1.12	4.47	5.24	#######	#####
F4-2 (2)	0.6475	1	NA	#VALUE!	#VALUE!	#VALUE!		
F4-3 (1)	1.6600	1	0.011	1.21	4.85	2.92	#######	#####
F4-3 (2)-	1.2327	1	NA	#VALUE!	#VALUE!	#VALUE!		
F5-0 (1)-	1.3699	1	0.014	1.50	6.02	4.39	4.32	0.11
F5-0 (2)	1.4196	1	0.014	1.50	6.02	4.24		
F5-2 (1)	1.7069	1	0.017	1.80	7.18	4.21	4.12	0.12
F5-2 (2)	1.4927	1	0.014	1.50	6.02	4.03		

F5-3 (1) -	0.9584	1	0.008	0.92	3.69	3.85		
							3.83	0.03
F5-3 (2)	1.3762	1	0.012	1.31	5.24	3.81		
C2-0 (1)	1.5129	1	0.041	4.13	16.50	10.91		
							11.97	1.50
C2-0 (2)	1.0881	1	0.035	3.54	14.17	13.03		
C2-2 (1)	1.8722	1	NA	#VALUE!	#VALUE!	#VALUE!		
							#######	#####
C2-2 (2)	1.7634	1	0.010	1.12	4.47	2.53		
C3-0 (1)	1.3404	1	0.016	1.70	6.80	5.07		
							#######	#####
C3-0 (2)	1.4339	1	NA	#VALUE!	#VALUE!	#VALUE!		
C3-1 (1)	1.4515	1	0.013	1.41	5.63	3.88		
							#######	#####
C3-1 (2)	1.1743	1	NA	#VALUE!	#VALUE!	#VALUE!		
C3-2 (1)	1.5375	1	0.011	1.21	4.85	3.16		
							3.24	0.12
C3-2 (2)	1.6911	1	0.013	1.41	5.63	3.33		
C4-0 (1)-	1.0124	1	NA	#VALUE!	#VALUE!	#VALUE!		
							#######	#####
C4-0 (2)	0.9055	1	0.012	1.31	5.24	5.79		

C4-2 (1)	1.0031	1	0.010	1.12	4.47	4.45		
							4.30	0.22
C4-2 (2)-	0.9852	1	0.009	1.02	4.08	4.14		
C5-0 (1)	1.2073	1	0.024	2.48	9.90	8.20		
							#######	#####
C5-0 (2)	1.0635	1	NA	#VALUE!	#VALUE!	#VALUE!		
C5-1 (1)	0.9645	1	NA	#VALUE!	#VALUE!	#VALUE!		
							#######	#####
C5-1 (2)	1.1362	1	0.017	1.80	7.18	6.32		
C5-2 (1)	1.2906	1	0.012	1.31	5.24	4.06		
							5.21	1.63
C5-2 (2)	1.9223	1	0.030	3.06	12.23	6.36		
blank		0						
Total chroform=4 ml.								

		Biomass on March 18, 1993 before backwashing						
Sample*	Weight (g)	Chloroform (ml)	Absorbance	Conc. (n mole)	TC. (n mole)	pl. n mole/g media	Average	S
F1-0 (1)	0.8966	1	0.001	0.24	0.97	1.08		
							1.59	0.72
F1-0 (2)	1.0194	1	0.004	0.53	2.14	2.10		
F1-2 (1)	0.6245	1	0.002	0.34	1.36	2.18		
							4.56	3.38
F1-2 (2)	0.6984	1	0.011	1.21	4.85	6.95		
F1-3 (1)	1.0535	1	0.010	1.12	4.47	4.24		
							4.56	0.45
F1-3 (2)	0.9160	1	0.010	1.12	4.47	4.88		
F2-0 (1)	1.0383	1	0.016	1.70	6.80	6.55		
							6.57	0.03
F2-0 (2)	1.0319	1	0.016	1.70	6.80	6.59		
F2-2 (1)	1.1445	1	0.012	1.31	5.24	4.58		
							6.11	2.17
F2-2 (2)	0.7876	1	0.014	1.50	6.02	7.64		
F2-3 (1)	0.9647	1	0.010	1.12	4.47	4.63		
							5.28	0.92
F2-3 (2)	0.8845	1	0.012	1.31	5.24	5.93		
F3-0 (1)	1.1937	1	0.008	0.92	3.69	3.09		

							5.30	3.12
F3-0 (2)-	1.0096	1	0.018	1.89	7.57	7.50		
F3-2 (1)	0.6184	1	0.009	1.02	4.08	6.59		
							4.59	2.84
F3-2 (2)	0.9783	1	0.005	0.63	2.52	2.58		
F3-3 (1)	1.1281	1	0.007	0.83	3.30	2.93		
							########	#####
F3-3 (2)	0.8138	1	NA	#VALUE!	#VALUE!	#VALUE!		
F4-0 (1)	0.8081	1	0.013	1.41	5.63	6.97		
							7.25	0.40
F4-0 (2)-	0.6438	1	0.011	1.21	4.85	7.54		
F4-2 (1)	0.7474	1	0.006	0.73	2.91	3.90		
							3.32	0.82
F4-2 (2)	0.6384	1	0.003	0.44	1.75	2.74		
F4-3 (1)	1.3570	1	0.000	0.15	0.58	0.43		
							0.42	0.01
F4-3 (2)-	1.4130	1	0.000	0.15	0.58	0.41		
F5-0 (1)-	1.4234	1	0.010	1.12	4.47	3.14		
							4.61	2.09
F5-0 (2)	1.1161	1	0.016	1.70	6.80	6.09		
F5-2 (1)	1.4451	1	0.013	1.41	5.63	3.90		

							4.38	0.69
F5-2 (2)	0.5180	1	0.005	0.63	2.52	4.87		
F5-3 (1) -	1.0196	1	na	#VALUE!	#VALUE!	#VALUE!		
							#######	#####
F5-3 (2)	0.9916	1	0.005	0.63	2.52	2.55		
C2-0 (1)	0.8483	1	0.031	3.16	12.62	14.88		
							12.50	3.37
C2-0 (2)	0.9020	1	0.022	2.28	9.13	10.12		
C2-2 (1)	1.3912	1	NA	#VALUE!	#VALUE!	#VALUE!		
							#######	#####
C2-2 (2)	1.0347	1	0.010	1.12	4.47	4.32		
C3-0 (1)	0.9447	1	0.014	1.50	6.02	6.37		
							4.97	1.99
C3-0 (2)	0.9261	1	0.007	0.83	3.30	3.56		
C3-1 (1)	0.9169	1	0.001	0.24	0.97	1.06		
							2.42	1.92
C3-1 (2)	0.8732	1	0.007	0.83	3.30	3.78		
C3-2 (1)	0.8654	1	0.001	0.19	0.78	0.90		
							0.98	0.11
C3-2 (2)	0.7362	1	0.001	0.19	0.78	1.06		
C4-0 (1)-	0.8848	1	0.008	0.92	3.69	4.17		

							3.85	0.45
C4-0 (2)	1.1520	1	0.009	1.02	4.08	3.54		
C4-2 (1)	1.2029	1	0.003	0.44	1.75	1.45		
							########	#####
C4-2 (2)-	0.9522		na	#VALUE!	#VALUE!	#VALUE!		
C5-0 (1)	0.8531	1	0.007	0.83	3.30	3.87		
							3.71	0.22
C5-0 (2)	0.9294	1	0.007	0.83	3.30	3.55		
C5-1 (1)	0.8020	1	0.004	0.53	2.14	2.66		
							2.52	0.20
C5-1 (2)	0.6111	1	0.001	0.24	1.46	2.38		
C5-2 (1)	0.9904	1	0.005	0.63	2.52	2.55		
							1.92	0.89
C5-2 (2)	1.0546	1	0.002	0.34	1.36	1.29		
blank		0						
Total chroform=4 ml.								

Biomass on March 18, 1993 after backwashing

Sample*	Weight (g)	Chloroform (ml)	Absorbance	Conc. (n mole)	TC. (n mole)	pl. n mole/g media	Average	S
F1-0 (1)	1.0567	1	0.003	0.44	1.75	1.65	1.62	0.05
F1-0 (2)	0.6126	1	0.001	0.24	0.97	1.58		
F1-2 (1)	1.1880	1	0.001	0.24	0.97	0.82	1.60	1.10
F1-2 (2)	0.8982	1	0.004	0.53	2.14	2.38		
F1-3 (1)	1.4551	1	0.004	0.53	2.14	1.47	1.37	0.14
F1-3 (2)	1.0688	1	0.002	0.34	1.36	1.27		
F2-0 (1)	0.9677	1	na	#VALUE!	#VALUE!	#VALUE!	#######	#####
F2-0 (2)	1.1029	1	0.011	1.21	4.85	4.40		
F2-2 (1)	1.3694	1	0.005	0.63	2.52	1.84	2.12	0.40
F2-2 (2)	1.2108	1	0.006	0.73	2.91	2.41		
F2-3 (1)	1.7287	1	0.004	0.53	2.14	1.24	1.13	0.15
F2-3 (2)	1.3307	1	0.002	0.34	1.36	1.02		
F3-0 (1)	1.0755	1	0.011	1.21	4.85	4.51		

							4.47	0.05
F3-0 (2)-	1.0943	1	0.011	1.21	4.85	4.44		
F3-2 (1)	1.2945	1	0.015	1.60	6.41	4.95	4.52	0.61
F3-2 (2)	0.9966	1	0.009	1.02	4.08	4.09		
F3-3 (1)	1.4105	1	0.009	1.02	4.08	2.89	2.61	0.40
F3-3 (2)	1.0855	1	0.005	0.63	2.52	2.33		
F4-0 (1)	0.6516	1	0.007	0.83	3.30	5.07	5.11	0.07
F4-0 (2)-	0.8650	1	0.010	1.12	4.47	5.16		
F4-2 (1)	0.5443	1	0.002	0.34	1.36	2.50	2.72	0.32
F4-2 (2)	0.9891	1	0.006	0.73	2.91	2.94		
F4-3 (1)	1.3579	1	na	#VALUE!	#VALUE!	#VALUE!	#######	#####
F4-3 (2)-	1.1155	1	na	#VALUE!	#VALUE!	#VALUE!		
F5-0 (1)-	1.2267	1	0.009	1.02	4.08	3.32	2.96	0.51
F5-0 (2)	1.4185	1	0.008	0.92	3.69	2.60		
F5-2 (1)	1.2439	1	0.011	1.21	4.85	3.90		

F5-2 (2)	1.3114	1	na	#VALUE!	#VALUE!	#VALUE!	#######	#####
F5-3 (1) -	1.2817	1	0.008	0.92	3.69	2.88		
F5-3 (2)	1.1043	1	na	#VALUE!	#VALUE!	#VALUE!	#######	#####
C2-0 (1)	0.9671	1	na	#VALUE!	#VALUE!	#VALUE!		
C2-0 (2)	1.1029	1	0.009	1.02	4.08	3.70	#######	#####
C2-2 (1)	1.4883	1	0.004	0.53	2.14	1.44		
C2-2 (2)	1.2062	1	0.003	0.44	1.75	1.45	1.44	0.01
C3-0 (1)	1.5051	1	0.011	1.21	4.85	3.23		
C3-0 (2)	1.2772	1	0.007	0.83	3.30	2.58	2.90	0.45
C3-1 (1)	1.1052	1	0.003	0.44	1.75	1.58		
C3-1 (2)	1.0187	1	na	#VALUE!	#VALUE!	#VALUE!	#######	#####
C3-2 (1)	0.9801	1	0.001	0.24	0.97	0.99		
C3-2 (2)	1.4860	1	0.002	0.34	1.36	0.91	0.95	0.05
C4-0 (1)-	0.9151	1	0.006	0.73	2.91	3.18		

							#######	#####
C4-0 (2)	1.4357	1	na	#VALUE!	#VALUE!	#VALUE!		
C4-2 (1)	1.1130	1	0.002	0.34	1.36	1.22	#######	#####
C4-2 (2)-	1.0195		na	#VALUE!	#VALUE!	#VALUE!		
C5-0 (1)	0.9286	1	0.008	0.92	3.69	3.97	3.60	0.53
C5-0 (2)	1.2664	1	0.009	1.02	4.08	3.22		
C5-1 (1)	0.9405	1	0.003	0.44	1.75	1.86	2.03	0.25
C5-1 (2)	1.1413	1	0.005	0.63	2.52	2.21		
C5-2 (1)	1.5317	1	0.001	0.20	0.80	0.52	#######	#####
C5-2 (2)	1.4581	1	na	#VALUE!	#VALUE!	#VALUE!		
blank	0							

Total chroform=4 ml.

Biomass on March 24, 1993

Sample*	Weight (g)	Chloroform (ml)	Absorbance	Conc. (n mole)	TC. (n mole)	pl. n mole/g media	Average	S
F1-0 (1)	0.6100	1	0.000	0.15	0.58	0.95	1.05	0.13
F1-0 (2)	0.8523	1	0.001	0.24	0.97	1.14		
F1-2 (1)	0.9881	1	0.003	0.44	1.75	1.77	1.75	0.03
F1-2 (2)	0.5621	1	0.001	0.24	0.97	1.73		
F1-3 (1)	0.7920	1	0.001	0.24	0.97	1.23	1.39	0.23
F1-3 (2)	0.6285	1	0.001	0.24	0.97	1.54		
F2-0 (1)	1.7637	1	0.010	1.12	4.47	2.53	2.44	0.12
F2-0 (2)	1.8956	1	0.010	1.12	4.47	2.36		
F2-2 (1)	1.2906	1	0.006	0.73	2.91	2.26	2.34	0.11
F2-2 (2)	1.5268	1	0.008	0.92	3.69	2.42		
F2-3 (1)	0.9799	1	0.002	0.34	1.36	1.39	1.55	0.23
F2-3 (2)	0.5647	1	0.001	0.24	0.97	1.72		
F3-0 (1)	0.7481	1	0.002	0.34	1.36	1.82		

							1.59	0.32
F3-0 (2)-	1.5628	1	0.004	0.53	2.14	1.37		
F3-2 (1)	1.1410	1	0.001	0.24	0.97	0.85	0.75	0.14
F3-2 (2)	0.8854	1	0.000	0.15	0.58	0.66		
F3-3 (1)	0.9150	1	0.001	0.24	0.97	1.06	1.18	0.16
F3-3 (2)	1.0522	1	0.002	0.34	1.36	1.29		
F4-0 (1)	0.6330	1	0.004	0.53	2.14	3.37	3.21	0.23
F4-0 (2)-	0.9562	1	0.006	0.73	2.91	3.05		
F4-2 (1)	1.0174	1	na	#VALUE!	#VALUE!	#VALUE!	#######	#####
F4-2 (2)	0.8921	1	na	#VALUE!	#VALUE!	#VALUE!		
F4-3 (1)	1.5247	1	0.003	0.44	1.75	1.15	#######	#####
F4-3 (2)-	1.1578	1	na	#VALUE!	#VALUE!	#VALUE!		
F5-0 (1)-	0.7584	1	0.003	0.44	1.75	2.30	2.39	0.12
F5-0 (2)	0.5487	1	0.002	0.34	1.36	2.48		
F5-2 (1)	1.4608	1	0.000	0.15	0.58	0.40		

F5-2 (2)	1.2568	1	na	#VALUE!	#VALUE!	#VALUE!	#######	#####
F5-3 (1) -	1.5944	1	0.000	0.15	0.58	0.37	0.43	0.10
F5-3 (2)	1.1598	1	0.000	0.15	0.58	0.50		
C2-0 (1)	0.5337	1	0.006	0.73	2.91	5.46	4.98	0.68
C2-0 (2)	1.5987	1	0.017	1.80	7.18	4.49		
C2-2 (1)	0.9810	1	0.004	0.53	2.14	2.18	2.25	0.10
C2-2 (2)	1.5874	1	0.008	0.92	3.69	2.32		
C3-0 (1)	1.1825	1	0.001	0.24	0.97	0.82	0.84	0.03
C3-0 (2)	1.5824	1	0.002	0.34	1.36	0.86		
C3-1 (1)	0.7194	1	0.000	0.15	0.58	0.81	0.89	0.12
C3-1 (2)	0.5986	1	0.000	0.15	0.58	0.97		
C3-2 (1)	0.8601	1	0.000	0.15	0.58	0.68	0.52	0.22
C3-2 (2)	1.5987	1	0.000	0.15	0.58	0.36		
C4-0 (1)-	0.8256	1	0.001	0.24	1.46	1.76		

							1.42	0.49
C4-0 (2)	0.5423	1	0.000	0.15	0.58	1.07		
C4-2 (1)	1.4690	1	na	#VALUE!	#VALUE!	#VALUE!	#######	#####
C4-2 (2)-	0.6489		na	#VALUE!	#VALUE!	#VALUE!		
C5-0 (1)	0.9588	1	0.006	0.73	2.91	3.04	2.78	0.37
C5-0 (2)	0.8476	1	0.004	0.53	2.14	2.52		
C5-1 (1)	0.8587	1	0.000	0.15	0.58	0.68	0.76	0.12
C5-1 (2)	0.6871	1	0.000	0.15	0.58	0.85		
C5-2 (1)	1.2809	1	na	#VALUE!	#VALUE!	#VALUE!	#######	#####
C5-2 (2)	0.6478	1	0.000	0.15	0.58	0.90		
blank		0						

Total chroform=4 ml.

Biomass on March 26, 1993

Sample*	Weight (g)	Chloroform (ml)	Absorbance	Conc. (n mole)	TC. (n mole)	pl. n mole/g media	Average	S
F1-0 (1)	0.8537	1	0.013	1.41	5.63	6.60	6.55	0.06
F1-0 (2)	0.5671	1	0.008	0.92	3.69	6.51		
F1-2 (1)	0.9671	1	0.022	2.28	9.13	9.44	9.65	0.30
F1-2 (2)	0.8473	1	0.020	2.09	8.35	9.85		
F1-3 (1)	1.1949	1	0.016	1.70	6.80	5.69	5.69	0.01
F1-3 (2)	0.5791	1	0.007	0.83	3.30	5.70		
F2-0 (1)	1.0066	1	0.018	1.89	7.57	7.52	7.66	0.19
F2-0 (2)	0.8725	1	0.016	1.70	6.80	7.79		
F2-2 (1)	0.9084	1	0.015	1.60	6.41	7.05	7.00	0.08
F2-2 (2)	0.9235	1	0.015	1.60	6.41	6.94		
F2-3 (1)	1.4490	1	0.012	1.31	5.24	3.62	3.73	0.16
F2-3 (2)	0.7581	1	0.006	0.73	2.91	3.84		
F3-0 (1)	0.8044	1	0.009	1.02	4.08	5.07		

							6.36	1.83
F3-0 (2)-	1.5976	1	0.030	3.06	12.23	7.66		
F3-2 (1)	1.3155	1	0.015	1.60	6.41	4.87	5.44	0.81
F3-2 (2)	0.5489	1	0.007	0.83	3.30	6.01		
F3-3 (1)	1.4305	1	0.016	1.70	6.80	4.75	4.79	0.06
F3-3 (2)	0.8435	1	0.009	1.02	4.08	4.83		
F4-0 (1)	0.3706	1	0.016	1.70	6.80	18.34	18.61	0.39
F4-0 (2)-	0.9562	1	0.045	4.51	18.06	18.89		
F4-2 (1)	1.0174	1	0.010	1.12	4.47	4.39	#######	#####
F4-2 (2)	0.8546	1	na	#VALUE!	#VALUE!	#VALUE!		
F4-3 (1)	0.8694	1	0.006	0.73	2.91	3.35	3.21	0.19
F4-3 (2)-	1.9574	1	0.014	1.50	6.02	3.08		
F5-0 (1)	0.5686	1	0.023	2.38	9.51	16.73	#######	#####
F5-0 (2)	0.8475	1	na	#VALUE!	#VALUE!	#VALUE!		
F5-2 (1)-	1.0483	1	0.011	1.21	4.85	4.63		

							4.49	0.20
F5-2 (2)	0.8472	1	0.008	0.92	3.69	4.35		
F5-3 (1) -	1.4735	1	0.004	0.53	2.14	1.45	1.47	0.03
F5-3 (2)	1.9457	1	0.006	0.73	2.91	1.50		
C2-0 (1)	0.5172	1	0.034	3.45	13.79	26.66	25.84	1.15
C2-0 (2)	0.9541	1	0.060	5.97	23.88	25.03		
C2-2 (1)	1.0132	1	0.027	2.77	11.07	10.92	10.92	#####
C2-2 (2)	1.0465	1	0.027	2.77	11.07	10.58		
C3-0 (1)	1.3855	1	0.100	9.85	39.42	28.45	27.05	1.98
C3-0 (2)	1.2341	1	0.080	7.91	31.65	25.65		
C3-1 (1)	0.6825	1	0.040	4.03	16.12	23.61	########	#####
C3-1 (2)	0.5478	1	na	#VALUE!	#VALUE!	#VALUE!		
C3-2 (1)	1.1264	1	0.033	3.35	13.40	11.89	10.84	1.48
C3-2 (2)	0.6542	1	0.015	1.60	6.41	9.79		
C4-0 (1)-	0.7528	1	0.017	1.80	7.18	9.54		

							9.76	0.31
C4-0 (2)	0.6421	1	0.015	1.60	6.41	9.98		
C4-2 (1)	1.5544	1	0.009	1.02	4.08	2.62	#######	#####
C4-2 (2)-	0.6543	1	na	#VALUE!	#VALUE!	#VALUE!		
C5-0 (1)	1.7611	1	0.013	1.41	5.63	3.20	3.56	0.52
C5-0 (2)	0.6425	1	0.005	0.63	2.52	3.93		
C5-1 (1)	0.9546	1	0.006	0.73	2.91	3.05	3.19	0.19
C5-1 (2)	0.7598	1	0.005	0.63	2.52	3.32		
C5-2 (1)	1.3094	1	0.008	0.92	3.69	2.82	2.76	0.09
C5-2 (2)	0.6487	1	0.003	0.44	1.75	2.69		
blank		0						

Total chroform=4 ml.

Biomass on March 29, 1993

Sample*	Weight (g)	Chloroform (ml)	Absorbance	Conc. (n mole)	TC. (n mole)	pl. n mole/g media	Average	S
F2-0 (1)	1.0615	1	0.110	10.83	43.30	40.79	37.66	4.42
F2-0 (2)	0.5791	1	0.050	5.00	20.00	34.54		
F2-2 (1)	0.8742	1	0.050	5.00	20.00	22.88	27.71	6.83
F2-2 (2)	0.6147	1	0.050	5.00	20.00	32.54		
F2-3 (1)	1.0300	1	0.000	0.15	0.58	0.57	0.83	0.38
F2-3 (2)	0.5312	1	0	0.15	0.58	1.10		
C2-0 (1)	0.9781	1	0.064	6.36	25.44	26.01	26.05	0.06
C2-0 (2)	0.9154	1	0.060	5.97	23.88	26.09		
C2-1' (1)	0.8595	1	0.005	0.63	2.52	2.94	2.94	0.00
C2-1' (2)	1.6521	1	0.011	1.21	4.85	2.94		
C2-2 (1)	0.8724	1	0.004	0.53	2.14	2.45	2.24	0.29
C2-2 (2)	1.6240	1	0.007	0.83	3.30	2.03		
blank		0						

Total chroform=4 ml.

Biomass on April 1, 1993

Sample*	Weight (g)	Chloroform (ml)	Absorbance	Conc. (n mole)	TC. (n mole)	pl. n mole/g media	Average	S
F1-0 (1)	1.0360	1	0.012	1.31	5.24	5.06	5.61	0.78
F1-0 (2)	0.5987	1	0.008	0.92	3.69	6.16		
F1-2 (1)	0.8164	1	0.012	1.31	5.24	6.42	6.68	0.37
F1-2 (2)	1.5390	1	0.026	2.67	10.68	6.94		
F1-3 (1)	1.4207	1	0.004	0.53	2.14	1.50	1.92	0.60
F1-3 (2)	0.5793	1	0.002	0.34	1.36	2.35		
F2-0 (1)	0.5731	1	0.009	1.02	4.08	7.12	6.90	0.31
F2-0 (2)	0.8431	1	0.013	1.41	5.63	6.68		
F2-2 (1)	1.2899	1	0.009	1.02	4.08	3.16	3.35	0.26
F2-2 (2)	0.9341	1	0.007	0.83	3.30	3.53		
F2-3 (1)	1.5933	1	0.005	0.63	2.52	1.58	1.83	0.35
F2-3 (2)	0.843	1	0.003	0.44	1.75	2.07		
F4-0 (1)	0.3927	1	0.023	2.38	9.51	24.23		

F4-0 (2)-	0.6218	1	0.046	4.61	18.45	29.67	26.95	3.85
F4-2 (1)	1.0937	1	0.018	1.89	7.57	6.92	7.31	0.55
F4-2 (2)	0.8319	1	0.015	1.60	6.41	7.70		
F4-3 (1)	1.4704	1	0.015	1.60	6.41	4.36	4.28	0.11
F4-3 (2)-	1.5231	1	0.015	1.60	6.41	4.21		
C2-0 (1)	0.6238	1	0.013	1.41	5.63	9.03	8.36	0.94
C2-0 (2)	0.7318	1	0.013	1.41	5.63	7.69		
C2-2 (1)	1.5109	1	0.018	1.89	7.57	5.01	5.05	0.05
C2-2 (2)	0.7259	1	0.008	0.92	3.69	5.08		
C4-0 (1)-	0.9450	1	0.013	1.41	5.63	5.96	6.08	0.18
C4-0 (2)	0.7821	1	0.011	1.21	4.85	6.21		
C4-2 (1)	1.4696	1	0.011	1.21	4.85	3.30	3.40	0.14
C4-2 (2)- blank	1.0548	1 0	0.008	0.92	3.69	3.50		

Total chroform=4 ml.

Appendix C

Pilot Plant Operational Data

Opertional Parameters

pH/Temp(C)/flowrate (m/h)	18-Feb	25-Feb	3-Mar	10-Mar	MAR.18
F1	8.01/9.00/10.01	7.49/8.1/10.65	*/*/10.33	7.70/9.2/10.01	8.09/9.9/10.01
F2	7.77/9.00/9.55	7.31/9.1/7.93	7.67/9.9/9.55	7.64/9.9/9.55	7.69/6.1
F3	7.88/10.3/4.48	7.23/10.0/4.71	7.65/10.4/4.48	7.65/10.4/4.71	8.07/10.9/4.71
F4	7.82/10.4/3.82	7.24/9.6/5.28	7.66/10.3/4.94	7.64/10.1/5.17	8.08/10.5/5.5
F5	7.87/8.2/9.13	7.23/8.2/10.12	7.65/8.8/9.98	7.68/8.9/9.98	8.04/9.4/9.55
C2	7.75/10.2/7.87	7.49/11.2/8.03	7.62/11.2/7.87	7.75/11.3/8.03	8.09/11.7/8.03
C3	7.93/12.1/3.76	7.53/11.5/3.76	7.72/12.2/4.68	7.82/12.2/3.89	8.22/12.3/12.3
C4	7.82/12.8/3.50	7.5/11.3/3.91	7.62/12.1/3.91	7.75/12/3.91	8.15/12.2/12.2
C5	7.84/10.10/7.85	7.24/11.2/7.97	7.66/10.5/7.97	7.74/10.2/7.97	8.09/11.1/11.1

pH/Temp(C)/flowrate (m/h)	24-Mar	26-Mar	29-Mar	1-Apr
F1	7.86/5.4	7.86/5.6	7.82/6.3	7.78/5.5
F2	7.69/6.1	7.69/6.1	7.72/6.4	7.76/7.8
F3	7.81/7.2/	7.81/12	7.80/7.8	7.72/8.0
F4	7.63/7.3	7.63/7.3	7.77/7.9	7.85/8.1
F5	7.87/5.1	7.87/5.1	7.89/5.6	7.84/5.9
C2	7.9/8.1	7.9/8.1	7.97/8.8	7.83/9.3
C3	8.07/10.1	8.07/10.1	8.2/10.2	8.08/10.6
C4	7.93/9.8	7.95/9.8	7.98/9.9	7.87/10.2
C5	7.91/7.8/	7.91/7.8	7.94/7.9/	7.88/7.9

Appendix D

Example Statistical Analysis

Paired t-test between F3-0 and F4-0

	FEB.25	MAR.3	MAR.10	MAR.18	MAR.24	MAR.26
F3-0	3.66	4.37	5.44	5.3	1.59	6.36
F4-0	4.13	6.92	6.62	7.25	3.21	12.22

'aired Two-Sample for Means

	Variable 1	*Variable 2*
Mean	4.453333	6.725
Variance	2.832147	9.92179
Observations	6	6
Pearson Correlation	0.864945	
Pooled Variance	4.58502	
thesized Mean Differ	0	
df	5	
t	-2.93929	
P(T<=t) one-tail	0.016142	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.032283	
t Critical two-tail	2.570578	

Paired t-tes between F3-2 and F4-2

	FEB.25	MAR.3	MAR.10	MAR.18	MAR.26
F3-2	2.65	3.58	3.88	4.59	5.44
F4-2	2.63	2.24	5.24	3.22	4.39

'aired Two-Sample for Means

	Variable 1	*Variable 2*
Mean	4.028	3.544
Variance	1.10777	1.55823
Observations	5	5
Pearson Correlation	0.495713	
Pooled Variance	0.651285	
thesized Mean Differ	0	
df	4	
t	0.92686	
P(T<=t) one-tail	0.20323	
t Critical one-tail	2.131846	
P(T<=t) two-tail	0.406461	
t Critical two-tail	2.776451	

Paired t-test between F3-3 and F4-3

	FEB.25	MAR.3	MAR.10	MAR.18	MAR.24	MAR.26
F3-3	2.5	3.41	1.45	2.93	1.18	4.79
F4-3	1.71	1.74	2.92	0.42	1.15	3.21

'aired Two-Sample for Means

	Variable 1	*Variable 2*
Mean	2.71	1.858333
Variance	1.76748	1.112137
Observations	6	6
Pearson Correlation	0.312319	
Pooled Variance	0.43788	
thesized Mean Differ	0	
df	5	
t	1.47371	
P(T<=t) one-tail	0.100279	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.200559	
t Critical two-tail	2.570578	

Paired t-test between C3-0 and C4-0

	FEB.18	FEB.25	MAR.3	MAR.10	MAR.18	MAR.24
C3-0	5.36	6.18	5.07	4.97	0.84	27.05
C4-0	7.03	3.95	5.79	3.85	1.42	9.76

'aired Two-Sample for Means

	Variable 1	*Variable 2*
Mean	8.245	5.3
Variance	88.37115	8.4208
Observations	6	6
Pearson Correlation	0.832805	
Pooled Variance	22.71828	
thesized Mean Differ	0	
df	5	
t	1.006625	
P(T<=t) one-tail	0.180158	
t Critical one-tail	2.015049	
P(T<=t) two-tail	0.360316	
t Critical two-tail	2.570578	

Paired t-test between C3-2 and C4-2

	FEB.18	FEB.25	MAR.3	MAR.10	MAR.18
C3-2	2.86	0.94	3.24	0.95	10.84
C4-2	0.76	3.29	4.3	1.45	2.62

aired Two-Sample for Means

	Variable 1	*Variable 2*
Mean	3.766	2.484
Variance	16.76378	2.00183
Observations	5	5
Pearson Correlation	0.095063	
Pooled Variance	0.550695	
thesized Mean Differ	0	
df	4	
t	0.682065	
P(T<=t) one-tail	0.266323	
t Critical one-tail	2.131846	
P(T<=t) two-tail	0.532647	
t Critical two-tail	2.776451	

		Linear regression (AOC input and nmol/g) in filters from Mar. 3 to 10								
		MAR.3	*Regression Statistics*							
F3-0	14.4	4.37								
F4-0	14.4	6.92		Multiple R	0.190238					
F3-0	61.52	5.44		R Square	0.036191					
F4-0	61.52	6.62		Adjusted I	-0.44571					
				Standard	1.404893					
				Observati	4					
			Analysis of Variance							
					df	*n of Squares*	*Mean Square*	*F*	*gnificance F*	
				Regressior	1	0.148225	0.148225	0.075099	0.809762	
				Residual	2	3.94745	1.973725			
				Total	3	4.095675				
					Coefficients	*andard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
				Intercept	5.527343	1.332055	4.149486	0.025433	-0.20403	11.25872
				x1	0.008171	0.029815	0.274042	0.801838	-0.12011	0.136455

				Linear regression (AOC input and nmol/g) in filters from Mar. 18 to Apr.1						
		MAR.3		*Regression Statistics*						
F3-0	9.96	1.59								
F4-0	9.96	3.21		Multiple R	0.076266					
F5-0	5.39	2.39		R Square	0.005816					
F3-0	82.82	6.36		Adjusted I	-0.24273					
F4-0	82.82	12.22		Standard	10.82416					
F4-0	12.06	26.95		Observati	6					
				Analysis of Variance						
					df	*m of Squares*	*lean Square*	*F*	*gnificance F*	
				Regressior	1	2.741838	2.741838	0.023402	0.885823	
				Residual	4	468.6499	117.1625			
				Total	5	471.3917				
					Coefficients	*andard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
				Intercept	8.127421	6.172386	1.316739	0.245047	-9.00991	25.26475
				x1	0.019484	0.127366	0.152977	0.884398	-0.33414	0.373111

				Linear regression (AOC input and nmol/g) in contactors						
		MAR.3	*Regression Statistics*							
C2-0	20.4	8.76								
C4-0	9.6	3.95		Multiple R	0.732424					
C2-0	48.76	11.97		R Square	0.536445					
C4-0	14.12	5.79		Adjusted I	0.494304					
C2-0	11.94	4.98		Standard	5.832957					
C3-0	7.03	0.84		Observati	13					
C4-0	5.87	1.42								
C5-0	5.62	2.78	*Analysis of Variance*							
C2-0	91.94	25.84			*df*	*m of Squares*	*Mean Square*	*F*	*gnificance F*	
C4-0	52.24	9.76		Regressior	1	433.1067	433.1067	12.72968	0.004412	
C2-0	23.58	26.05		Residual	11	374.2573	34.02339			
C2-0	23.76	8.36		Total	12	807.364				
C4-0	16.06	6.08								
					Coefficients	*andard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
				Intercept	2.842415	2.35893	1.20496	0.251445	-2.34956	8.034387
				x1	0.240628	0.067443	3.567867	0.003868	0.092187	0.389069

Linear regression (AOC input and nmol/cm2) in filters from Mar. 3 to10

MAR.3

F3-0	14.4	3.63
F4-0	14.4	3.05
F3-0	61.52	4.52
F4-0	61.52	2.91

Regression Statistics

Multiple R	0.296013
R Square	0.087624
Adjusted I	-0.36856
Standard	0.855643
Observati	4

Analysis of Variance

	df	m of Squares	lean Square		F ignificance F
Regressior	1	0.140625	0.140625	0.192078	0.703987
Residual	2	1.46425	0.732125		
Total	3	1.604875			

	Coefficients	andard Error	t Statistic	P-value	Lower 95%	Upper 95%
Intercept	3.225349	0.811281	3.975685	0.028459	-0.26526	6.716063
x1	0.007958	0.018159	0.438267	0.690832	-0.07017	0.08609

		Linnear regression (AOC input and nmol/cm2) in filters from Mar. 18 to Apr. 1							
		MAR.24	*Regression Statistics*						
F3-0	9.96	1.32							
F4-0	9.96	1.41		Multiple R	0.185581				
F5-0	5.39	1.98		R Square	0.03444				
F3-0	82.82	5.28		Adjusted I	-0.20695				
F4-0	82.82	5.38		Standard	4.436939				
F4-0	12.06	11.86		Observati	6				
			Analysis of Variance						
					df	*m of Squares*	*Mean Square*	*F*	*gnificance F*
				Regressior	1	2.80877	2.80877	0.142675	0.724824
				Residual	4	78.74571	19.68643		
				Total	5	81.55448			
					Coefficients	*andard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
				Intercept	3.871089	2.530127	1.529998	0.186571	-3.15368	10.89586
				x1	0.019721	0.052209	0.377724	0.721139	-0.12523	0.164676

		Linear regression (AOC input and nmol/cm2) in contactors								
		MAR.3	*Regression Statistics*							
C2-0	20.4	3.77								
C4-0	9.6	1.7		Multiple R	0.73254					
C2-0	48.76	5.15		R Square	0.536615					
C4-0	14.12	2.49		Adjusted I	0.494489					
C2-0	11.94	2.14		Standard	2.507404					
C3-0	7.03	0.36		Observati	13					
C4-0	5.87	0.61								
C5-0	5.62	1.2	*Analysis of Variance*							
C2-0	91.94	11.11			*df*	*n of Squares*	*Mean Square*	*F*	*gnificance F*	
C4-0	52.24	4.2		Regressior	1	80.08703	80.08703	12.73836	0.004402	
C2-0	23.58	11.2		Residual	11	69.15785	6.287077			
C2-0	23.76	3.6		Total	12	149.2449				
C4-0	16.06	2.61								
					Coefficients	*andard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
				Intercept	1.222962	1.01403	1.206042	0.251042	-1.0089	3.454827
				x1	0.103474	0.028992	3.569084	0.003859	0.039663	0.167284

Linear regression (AOC removal and nmol/g) in filters from Mar.3 to 10

	MAR.3		Regression Statistics							
F4avg	3.633333	4.8								
F4-0	4.926667	47.4		Multiple R	0.249914					
				R Square	0.062457					
				Adjusted I	-0.12505					
			Regression Statistics							
				Multiple R	1					
				R Square	1					
				Adjusted I	#DIV/0!					
				Standard	0					
				Observati	2					
			Analysis of Variance							
					df	m of Squares	Mean Square	F	gnificance F	
				Regressior	1	907.38	907.38	0	#NUM!	
				Residual	0	3.31E-27	#DIV/0!			
				Total	1	907.38				
					Coefficients	andard Error	t Statistic	P-value	Lower 95%	Upper 95%
				Intercept	-114.875	0	#DIV/0!	#DIV/0!	#NUM!	#NUM!
				x1	32.93813	0	#DIV/0!	#DIV/0!	#NUM!	#NUM!

Linear regression (AOC removal and nmol/g) in filters from Mar.18 to Apr.1

F3-0	1.173333	12.59	
F4-0	2.18	12.84	
F5-0	1.073333	3.69	
F4-0	6.606667	3.79	
F4-0	12.84667	-4	

Regression Statistics

Multiple R	0.823986
R Square	0.678953
Adjusted !	0.571937
Standard	4.628623
Observati	5

Analysis of Variance

	df	*n of Squares*	*lean Square*	*F*	*gnificance F*
Regressior	1	135.9238	135.9238	6.344422	0.086267
Residual	3	64.27244	21.42415		
Total	4	200.1963			

	Coefficients	*andard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	11.2976	3.013284	3.749264	0.01996	1.707975	20.88722
x1	-1.15486	0.458493	-2.51881	0.065439	-2.61399	0.304272

		Linear regression (AOC removal and nmol/g) in contactors							
	MAR.3		*Regression Statistics*						
C2avg	5.47	18.1							
C4avg	3.62	5.1		Multiple R	0.827673				
C2-0	7.25	43.93		R Square	0.685042				
C4-0	5.045	5.11		Adjusted I	0.656409				
C2-0	3.615	7.03		Standard	14.49256				
C3-0	0.68	3.39		Observati	13				
C4-0	1.42	-2.12							
C5-0	1.84	-2.12	*Analysis of Variance*						
C2-0	18.38	85			*df*	*n of Squares*		*F*	*gnificance F*
C4-0	6.19	43.91		Regressior	1	5025.132	23.92528	0.000478	
C2-0	14.145	22.29		Residual	11	2310.379			
C2-0	6.705	19.17		Total	12	7335.511			
C4-0	4.74	10.5							
					Coefficients	*andard Error*	*P-value*	*Lower 95%*	*Upper 95%*
				Intercept	-4.86134	6.471255	0.466997	-19.1045	9.381801
				x1	4.076959	0.833504	0.000371	2.242428	5.911491

		Linear regression (AOC removal and nmol/cm2) in filters from Mar.3 to10								
	MAR.3		*Regression Statistics*							
F4avg	2.79	4.8								
F4-0	3.59	47.4		Multiple R	0.249914					
				R Square	0.062457					
				Adjusted I	-0.12505					
			Regression Statistics							
				Multiple R	1					
				R Square	1					
				Adjusted I	#DIV/0!					
				Standard	0					
				Observati	2					
			Analysis of Variance							
					df	*m of Squares*	*Mean Square*	*F*	*gnificance F*	
				Regressior	1	907.38	907.38	0	#NUM!	
				Residual	0	1.08E-26	#DIV/0!			
				Total	1	907.38				
					Coefficients	*andard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
				Intercept	-143.767	0	#DIV/0!	#DIV/0!	#NUM!	#NUM!
				x1	53.25	0	#DIV/0!	#DIV/0!	#NUM!	#NUM!

Linear regression (AOC removal and nmol/cm2) in filter from Mar.10 to Apr.1

F3-0	1.52	12.59	
F4-0	1.55	12.84	
F5-0	1.31	3.69	
F4-0	5.03	3.79	
F4-0	9.05	-4	

Regression Statistics

Multiple R	0.838412
R Square	0.702934
Adjusted I	0.603912
Standard	4.452398
Observati	5

Analysis of Variance

	df	*n of Squares*	*Mean Square*	*F*	*gnificance F*
Regressior	1	140.7247	140.7247	7.098761	0.076056
Residual	3	59.47154	19.82385		
Total	4	200.1963			

	Coefficients	*andard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	12.2765	3.147451	3.900459	0.017535	2.259899	22.29311
x1	-1.75907	0.660226	-2.66435	0.056135	-3.86021	0.342063

		Linear regression (AOC removal and nmol/cm2) in all contactors							
	MAR.3		*Regression Statistics*						
C2avg	2.35	18.1							
C4avg	1.56	5.1		Multiple R	0.828857				
C2-0	3.12	43.93		R Square	0.687004				
C4-0	2.17	5.11		Adjusted I	0.658549				
C2-0	1.56	7.03		Standard	14.44736				
C3-0	0.32	3.39		Observati	13				
C4-0	0.61	-2.12							
C5-0	0.64	-2.12	*Analysis of Variance*						
C2-0	7.9	85			*df*	*n of Squares*		*F gnificance F*	
C4-0	2.66	43.91		Regressior	1	5039.522	24.14416	0.000461	
C2-0	6.08	22.29		Residual	11	2295.989			
C2-0	2.88	19.17		Total	12	7335.511			
C4-0	2.04	10.5							
					Coefficients	*andard Error*	*P-value*	*Lower 95%*	*Upper 95%*
				Intercept	-4.72406	6.423553	0.476207	-18.8622	9.414088
				x1	9.463052	1.925862	0.000358	5.224256	13.70185

		Linear regression (thickness and nmol/g) in filters									
mar.3			BIOMASS	*Regression Statistics*							
	0.15	1.31	4.760032								
	0.1	2.83	4.737852		Multiple R	0.695838					
	0.25	2.51	4.80439		R Square	0.48419					
	0.6	4.37	4.959644		Adjusted I	0.474271					
	0.2	3.58	4.782211		Standard	3.834839					
	0.2	3.41	4.782211		Observati	54					
	0.5	6.92	4.915286								
	0.25	2.24	4.80439	*Analysis of Variance*							
	0.4	1.74	4.870928			*df*	*n of Squares*	*Mean Square*	*F*	*gnificance F*	
	1.2	2.73	5.225795		Regressior	1	717.8339	717.8339	48.81235	5.2E-09	
	0.5	0.8	4.915286		Residual	52	764.7116	14.70599			
	0.2	0.59	4.782211		Total	53	1482.545				
mar.10	0.2	4.66	4.782211			*Coefficients*	*andard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
	0	5.88	4.693494								
	0.5	5.39	4.915286		Intercept	4.693494	0.537495	8.732161	7.72E-12	3.614932	5.772056
	0.2	6.38	4.782211		x1	0.443584	0.063491	6.986583	4.74E-09	0.31618	0.570987
	0.2	5.44	4.782211								
	0	3.88	4.693494								
	0.5	6.62	4.915286								
	1	5.24	5.137078								
	0	4.32	4.693494								
	0	4.12	4.693494								
mar.18b	0.5	1.59	4.915286								
	0.625	4.56	4.970734								
	0	6.57	4.693494								
	0	6.11	4.693494								
	0.1	5.3	4.737852								
	0	4.59	4.693494								
	10	7.25	9.129334								
	5	3.22	6.911414								

	2	4.61	5.580662								
	0	4.38	4.693494								
mar.18a	0.5	1.62	4.915286								
	0.1	1.6	4.737852								
	0.9	4.4	5.09272								
	0.25	2.12	4.80439								
	0.5	4.47	4.915286								
	0.25	4.52	4.80439								
	0.1	5.11	4.737852								
	0	2.72	4.693494								
	0.25	2.96	4.80439								
	0	3.9	4.693494								
mar.26	0.25	7.66	4.80439								
	0	7	4.693494								
	0.5	6.36	4.915286								
	4	18.3	6.46783								
	1	4.39	5.137078								
	4	16.73	6.46783								
	0	4.49	4.693494								
mar.29	60	27.71	31.30853								
Apr.1	0	5.61	4.693494								
	1	6.9	5.137078								
	0.5	3.35	4.915286								
	10	26.95	9.129334								

Linear regression (thickness and nmol/g) in ozonated contactors

		BM	BM
c3-0	0	6.18	3.954658
c3-1	0	5.56	3.954658
c4-0	5	3.95	9.099578
c4-2	5	3.29	9.099578
c2-0	2	12	6.012626
c3-1	0	3.88	3.954658
c4-0	2.5	5.79	6.527118
c2-2	5	4.32	9.099578
c3-0	0	4.97	3.954658
c4-2	5	1.45	9.099578
c2-0	0.1	3.7	4.057556
c3-0	0	2.9	3.954658
c2-2	4	10.9	8.070594
c4-0	0.4	9.76	4.366252
c4-2	0	2.62	3.954658
c2-0	16	26.05	20.4184
c2-1	0.1	2.94	4.057556
c2-0	1	8.36	4.983642

Regression Statistics

Multiple R	0.713417
R Square	0.508964
Adjusted I	0.478275
Standard	4.116056
Observati	18

Analysis of Variance

	df	m of Squares	lean Square	F	gnificance F
Regressior	1	280.9681	280.9681	16.5842	0.000886
Residual	16	271.0707	16.94192		
Total	17	552.0388			

	Coefficients	andard Error	t Statistic	P-value	Lower 95%	Upper 95%
Intercept	3.954658	1.166187	3.391102	0.003474	1.482454	6.426863
x1	1.028984	0.252674	4.07237	0.000793	0.493338	1.56463

		Linear regression (thickness and nmol/g) in contactors									
		BM	BM	*Regression Statistics*							
c3-0	0	6.18	2.958007								
c3-1	0	5.56	2.958007		Multiple R	0.739758					
c4-0	5	3.95	8.764392		R Square	0.547242					
c4-2	5	3.29	8.764392		Adjusted I	0.528377					
c5-0	1.6	0.59	4.81605		Standard	4.178714					
c5-2	0	1.42	2.958007		Observati	26					
c2-0	2	12	5.280561								
c3-1	0	3.88	2.958007	*Analysis of Variance*							
c4-0	2.5	5.79	5.8612			*df*	*n of Squares*	*lean Square*		*F gnificance F*	
c5-0	10	8.2	14.57078		Regressior	1	506.5357	506.5357	29.00847	1.57E-05	
c5-1	2	6.32	5.280561		Residual	24	419.0795	17.46165			
c5-2	2	5.21	5.280561		Total	25	925.6152				
c2-2	5	4.32	8.764392								
c3-0	0	4.97	2.958007			*Coefficients*	*andard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
c4-2	5	1.45	8.764392								
c5-0	2	3.71	5.280561		Intercept	2.958007	1.061203	2.78741	0.010001	0.767793	5.148222
c2-0	0.1	3.7	3.074135		x1	1.161277	0.215612	5.385951	1.38E-05	0.716275	1.606279
c3-0	0	2.9	2.958007								
c2-2	4	10.9	7.603115								
c3-1	10	23.6	14.57078								
c4-0	4	9.76	7.603115								
c4-2	4	2.62	7.603115								
c5-0	0	3.65	2.958007								
c2-0	16	26.05	21.53844								
c2-1	0.1	2.94	3.074135								
c2-0	1	8.36	4.119284								

		Linear regression (thickness and nmol/cm3) in ozonated filters								
	n mol/cm3	Mar.3	n mol/cm2	*Regression Statistics*						
F2-0	1.09	0.15	0.13							
F2-3	3.66	0.25	0.23	Multiple R	0.2409					
F3-0	3.63	0.6	0.43	R Square	0.058					
F3-3	4.98	0.2	0.31	Adjusted R Square	0.0057					
F4-0	3.04	0.5	0.23	Standard Error	0.2315					
F4-3	2.54	0.4	0.16	Observations	20					
F2-0	4.47	0.5	0.53							
F3-0	4.52	0.2	0.53	*Analysis of Variance*						
F4-0	2.91	0.5	0.22		*df*	*f Squares*	*n Square*	*F*	*ficance F*	
F2-0	5.45	0	0.64	Regression	1	0.0595	0.059	1.11	0.3062	
F3-0	4.40	0.1	0.52	Residual	18	0.9649	0.054			
F4-0	3.19	10	0.24	Total	19	1.0244				
F2-0	2.03	0.9	0.24							
F3-0	1.32	0.5	0.16		*Coefficients*	*tard Error*	*t Statistic*	*P-value*	*ower 95%*	*per 95%*
F4-0	1.41	0.1	0.11							
F2-0	6.36	0.25	0.75	Intercept	0.3723	0.0584	6.376	0	0.2496	0.49
F3-0	5.28	0.5	0.62	x1	0.0185	0.0176	1.053	0.31	-0.018	0.06
F4-0	5.38	4	0.40							
F2-0	5.73	1	0.68							
F4-0	11.86	10	0.89							

Linear regression (thickness and nmol/cm3) in filters

	n mol/c	Mar.3		Regression Statistics						
F2-0	1.09	0.15	0.13							
F2-3	3.66	0.25	0.23	Multiple R	0.3112					
F3-0	3.63	0.6	0.43	R Square	0.0968					
F3-3	4.98	0.2	0.31	Adjusted R Square	0.0646					
F4-0	3.04	0.5	0.23	Standard Error	2.475					
F4-3	2.54	0.4	0.16	Observations	30					
F5-0	2.27	1.2	0.27							
F5-3	0.86	0.2	0.05	Analysis of Variance						
F1-0	3.87	0.2	0.46		df	f Squares	n Square	F	ficance F	
F2-0	4.47	0.5	0.53	Regression	1	18.387	18.39	3	0.0942	
F3-0	4.52	0.2	0.53	Residual	28	171.51	6.126			
F4-0	2.91	0.5	0.22	Total	29	189.9				
F5-0	3.59	0	0.42							
F1-0	1.32	0.5	0.16		Coefficients	ard Error	t Statistic	P-value	ower 95%	per 95%
F2-0	5.45	0	0.64							
F3-0	4.40	0.1	0.52	Intercept	0.2797	0.7499	0.373	0.71	-1.257	1.82
F4-0	3.19	10	0.24	x1	2.5192	1.454	1.733	0.09	-0.459	5.5
F5-0	3.83	2	0.45							
F1-0	0.87	0.5	0.10							
F2-0	2.03	0.9	0.24							
F3-0	1.32	0.5	0.16							
F4-0	1.41	0.1	0.11							
F5-0	1.98	0.25	0.23							
F2-0	6.36	0.25	0.75							
F3-0	5.28	0.5	0.62							
F4-0	5.38	4	0.40							
F5-0	13.89	4	1.64							
F1-0	4.66	0	0.55							
F2-0	5.73	1	0.68							
F4-0	11.86	10	0.89							

Linear regression (thickness and nmol/cm3) in ozonated contactors

	n mol/c	Mar.3			Regression Statistics						
C3-0	2.66	0	0.15	0.1365							
C3-1	2.39	0	0.20	0.1365	Multiple R	0.6775					
C4-0	1.70	5	0.10	0.322	R Square	0.4591					
C4-2	1.41	5	0.21	0.322	Adjusted R Square	0.4272					
C2-0	5.15	2	0.29	0.2107	Standard Error	0.1693					
C3-1	1.67	0	0.14	0.1365	Observations	19					
C4-0	2.49	2.5	0.14	0.2293							
C4-2	1.85	0.2	0.28	0.1439	Analysis of Variance						
C2-2	1.86	5	0.28	0.322		df	f Squares	n Square	F	ficance F	
C3-0	2.14	0	0.12	0.1365	Regression	1	0.4137	0.414	14.4	0.0014	
C4-2	0.62	5	0.09	0.322	Residual	17	0.4875	0.029			
C2-0	2.14	0.1	0.12	0.1402	Total	18	0.9012				
C3-0	0.36	0	0.02	0.1365							
C2-2	4.70	4	0.70	0.2849		Coefficients	tard Error	t Statistic	P-value	ower 95%	per 95%
C3-1	10.15	10	0.85	0.5075							
C4-0	4.20	4	0.24	0.2849	Intercept	0.1365	0.0508	2.687	0.02	0.0293	0.24
C4-2	1.13	4	0.17	0.2849	x1	0.0371	0.0098	3.798	0	0.0165	0.06
C2-0	11.20	16	0.64	0.7301							
C2-0	3.59	1	0.20	0.1736							

		Linear regression (thickness and nmol/cm3) in contactors									
	n mol/c	Mar.3			*Regression Statistics*						
C3-0	2.66	0	0.15	0.1282							
C3-1	2.39	0	0.20	0.1282	Multiple R	0.6258					
C4-0	1.70	5	0.10	0.2927	R Square	0.3917					
C4-2	1.41	5	0.21	0.2927	Adjusted R Square	0.3663					
C5-0	0.25	1.6	0.01	0.1808	Standard Error	0.1621					
C5-2	0.61	0	0.09	0.1282	Observations	26					
C2-0	5.15	2	0.29	0.194							
C3-1	1.67	0	0.14	0.1282	*Analysis of Variance*						
C4-0	2.49	2.5	0.14	0.2105		*df*	*f Squares*	*n Square*	*F*	*ficance F*	
C4-2	1.85	0.2	0.28	0.1348	Regression	1	0.4059	0.406	15.5	0.0006	
C5-0	3.53	10	0.20	0.4572	Residual	24	0.6304	0.026			
C5-1	2.72	2	0.23	0.194	Total	25	1.0363				
C5-2	2.24	2	0.34	0.194							
C2-2	1.86	5	0.28	0.2927		*Coefficients*	*tard Error*	*t Statistic*	*P-value*	*ower 95%*	*per 95%*
C3-0	2.14	0	0.12	0.1282							
C4-2	0.62	5	0.09	0.2927	Intercept	0.1282	0.0412	3.112	0	0.0432	0.21
C5-0	1.60	2	0.09	0.194	x1	0.0329	0.0084	3.931	0	0.0156	0.05
C2-0	2.14	0.1	0.12	0.1315							
C3-0	0.36	0	0.02	0.1282							
C2-2	4.70	4	0.70	0.2598							
C3-1	10.15	10	0.85	0.4572							
C4-0	4.20	4	0.24	0.2598							
C4-2	1.13	4	0.17	0.2598							
C5-0	1.57	0	0.09	0.1282							
C2-0	11.20	16	0.64	0.6546							
C2-0	3.59	1	0.20	0.1611							